Author:
O'Connell, Yvonne

Title:
Synthesis of putative polyketide precursors and intermediates

Synthesis of Putative Polyketide Precursors and Intermediates

Yvonne O'Connell

A thesis submitted to the University of Bristol in accordance with the requirements for the degree of Doctor of Philosophy in the Faculty of Science.

University of Bristol
School of Chemistry
Cantock's Close
Bristol
BS8 1TS

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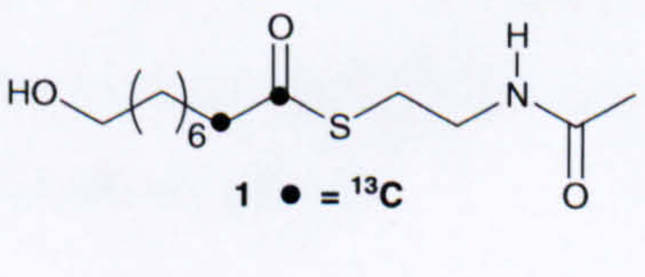
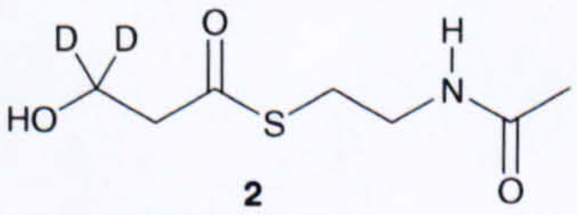
Abstract

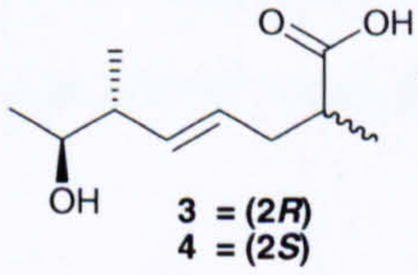
The work described in this thesis pertains to three separate collaborative projects, each involving the synthesis of putative precursors and intermediates to polyketides - the pseudomonic acids (mupirocin), strobilurin A and alternapyrone.

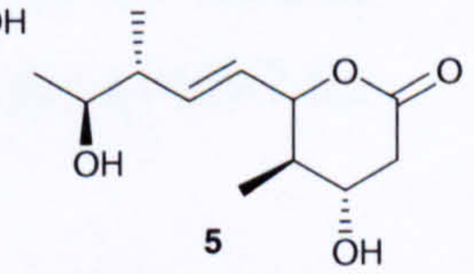
Following a general introduction, chapter 2 describes isotopic labelling studies conducted on the antibacterial pseudomonic acids. A growth production study of *Pseudomonas fluorescens* determined the optimal conditions for feeding studies. Thiol esters **1** and **2** were prepared and fed to the bacteria. No incorporation of isotopic label into pseudomonic acid was observed for either of these substrates.

Chapter 3 discusses the synthesis of mupirocin metabolites isolated from mutant strains of *P. fluorescens*. Mupiric acid **3** and its C-2 epimer **4** were prepared using a cross metathesis approach to unequivocally assign the C-2 centre as the *R* configuration. A flexible route for the synthesis of lactone **5**, the proposed DH4 mutant metabolite, has also been developed. The core was prepared *via* a stereoselective Prins cyclisation. Some unexpected side products resulting from this key step, give further insight into the mechanism of the Prins reaction.



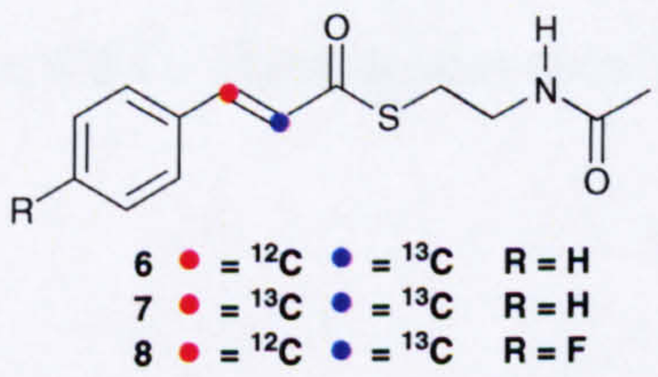


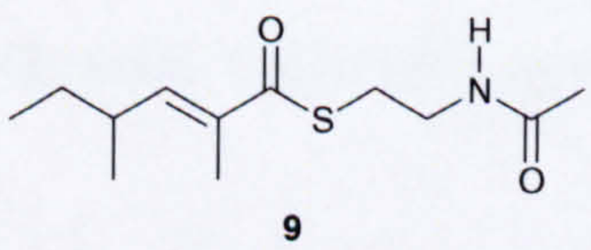


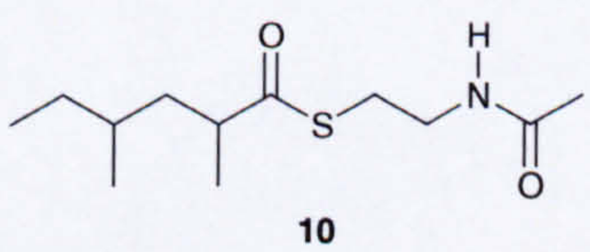


Chapter 4 summarises the synthesis of putative strobilurin precursors, thiol esters **6** and **7**. These were then fed to *Strobilurus tenacellus* by Zeeck and Thormann. Incorporation patterns have shown that the C-2–C-3 bond is cleaved during strobilurin biosynthesis. An attempted precursor-directed biosynthesis, employing thiol ester **8** as the unnatural substrate, is also discussed.

The preparation of putative alternapyrone precursors, thiol esters **9** and **10** is discussed in chapter 5. Thiol ester **9** has been fed to partially purified cell-free extracts of the genetically engineered PKSN by Fujii and Kasahara and showed positive incorporation.







Acknowledgements

First and foremost, I would like to thank my supervisors Prof. Chris Willis and Prof. Tom Simpson for giving me the opportunity to come to Bristol. Chris, there are no words to express how grateful I am for all your help and support over the past three years. I don't know how you remain so enthusiastic and optimistic all the time but it's something I aspire to. Tom, thanks for your advice, your patience, and the bit of craic!

I would also like to thank Dr. Russell Cox for his stringent correction of my annual reports. His suggestions have been invaluable and have helped shape this thesis into something I can be proud of. Thanks also to James, Jennifer and Peter for proof reading.

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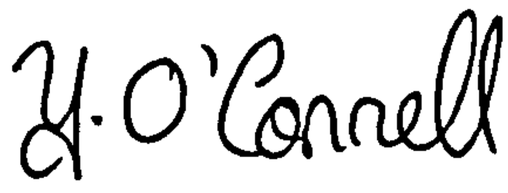
I have been very fortunate to have made lots of wonderful friends in Bristol. Thanks to the CLW group past and present, especially my tea buddies Angela, Dave, Jon, Lorraine, Natalie, Mandy and Peter, Conor for helping me settle in those first few weeks and Carlo for his frequent advice. Thanks also to Ursula, Sam, Kat, Val, Liam, Chris and Rob for being some of the best friends I've ever had and for some great nights out! I would also like to thank my friends at home in Ireland who have made a valiant effort to keep in touch over the past few years - especially May, who has even crossed the pond on occasion!

Finally, I would like to thank my parents for their eternal love and support (and for the silly o'clock airport runs!). Go raibh míle maith agaibh.

Author's Declaration

The work described in this thesis was carried out in the School of Chemistry, University of Bristol under the supervision of Prof. C. L. Willis and Prof. T. J. Simpson between October 2004 and September 2007. Except where indicated by reference, the work is original and has not been submitted for any other degree. The views expressed in this thesis are those of the author and in no way represent those of the University of Bristol.



Yvonne O'Connell

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Abbreviations

Ac	acetyl
ACP	acyl carrier protein
$[\alpha]_D$	specific optical rotation
AHL	*N*-acylhomoserine lactone
app.	apparent
Ar	aromatic
AT	acyl transferase
ATP	adenosine triphosphate
ax	axial
Bn	benzyl
br	broad
c	concentration
CFE	cell free extract
CI	chemical ionisation
CoA	coenzyme A
conc.	concentration
COSY	correlation spectroscopy
d	doublet
D	deuterium
Δ	heat
Δ	gene knockout
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
δ_C	^{13}C chemical shift
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEPT	distortionless enhancement by polarization transfer
δ_H	^{1}H chemical shift
DH	dehydratase
DHP	dihydropyran
DIBAL-H	diisobutylaluminium hydride
DIPHOS	1,2-bis(diphenylphosphino)ethane

DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMP	Dess-Martin periodinane
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2-(1H)-pyrimidinone
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EDCI	1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide
EI	electron impact
eq	equatorial
ER	enoyl reductase
ESI	electrospray ionisation
FAD	flavin adenine dinucleotide
FAS	fatty acid synthase
$FMNH_2$	reduced flavin mononucleotide
FT	Fourier transform
GEB	3-β-[2-(dimethylamino)ethoxy]androst-5-en-17-one
HMQC	heteronuclear multiple quantum coherence
HMBC	heteronuclear multiple bond correlation
HMDS	hexamethyldisilazide
HMG	3-hydroxy-3-methylglutaryl
HMPA	hexamethylphosphoramide
HPLC	high performance liquid chromatography
HSNAC	*N*-acetylcysteamine
IR	infra red
J	coupling constant
KR	ketoreductase
KS	ketosynthase
λ	wavelength
LC-MS	liquid chromatography mass spectrometry
LDA	lithium diisopropylamide
liq.	liquid
lit.	literature
m	multiplet

M	molarity
M^+	molecular ion
MeT	methyl transferase
MH^+	protonated molecular ion
MNa^+	sodiated molecular ion
MOM	methoxymethyl ether
m.p.	melting point
MS	mass spectrometry
MT	malonyl transferase
m/z	mass to charge ratio
NAC	*N*-acetylcysteamine
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NCIMB	National Collections of Industrial, Marine and Food Bacteria
NMR	nuclear magnetic resonance
Nu	nucleophile
oct.	octet
ORF	open reading frame
PAL	phenylalanine ammonia lyase
PCC	pyridinium chlorochromate
Piv	pivaloyl
PKS	polyketide synthase
q	quartet
quint.	quintet
RNA	ribonucleic acid
tRNA	transfer ribonucleic acid
R.T.	room temperature
s	singlet
SAM	*S*-adenosyl methionine
sept.	septet
sext.	sextet
t	triplet
TAL	triacetic acid lactone
TBAI	tetra-*n*-butylammonium iodide

TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
TE	thiol esterase
TES	triethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
T.L.C.	thin layer chromatography
TMS	trimethylsilyl
*p*TSA	*para*-toluenesulfonic acid
υ_{max}	wavenumber
UV	ultra violet

Contents

Chapter 1

Introduction

1.1 Polyketide Biosynthesis

The polyketides are a diverse group of naturally occurring secondary metabolites related by their common biogenesis. Predominantly derived from acetate, they encompass a wide spectrum of compounds of varied structure, functionality and biological activity (Figure 1).[1,2,3]

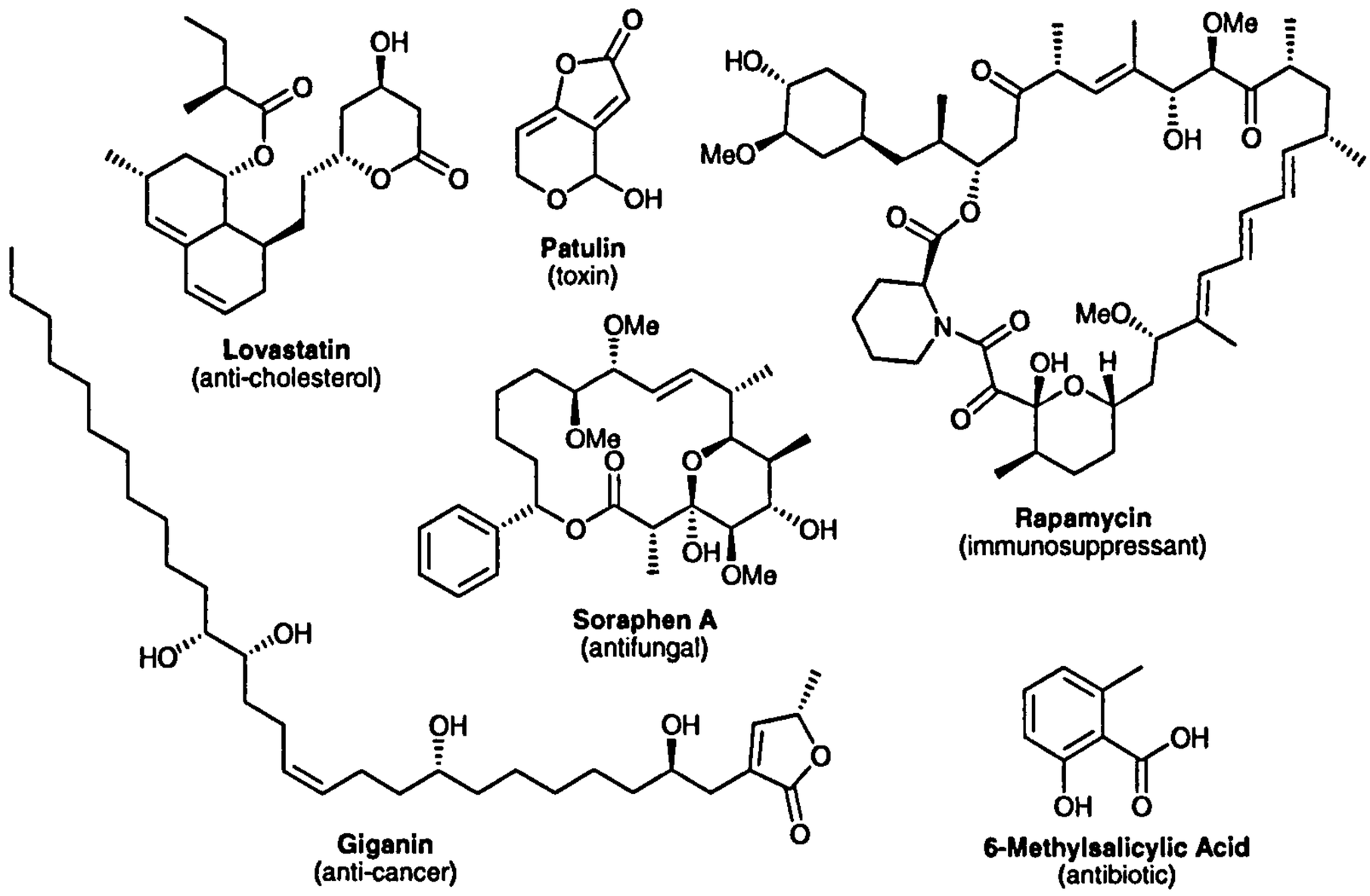
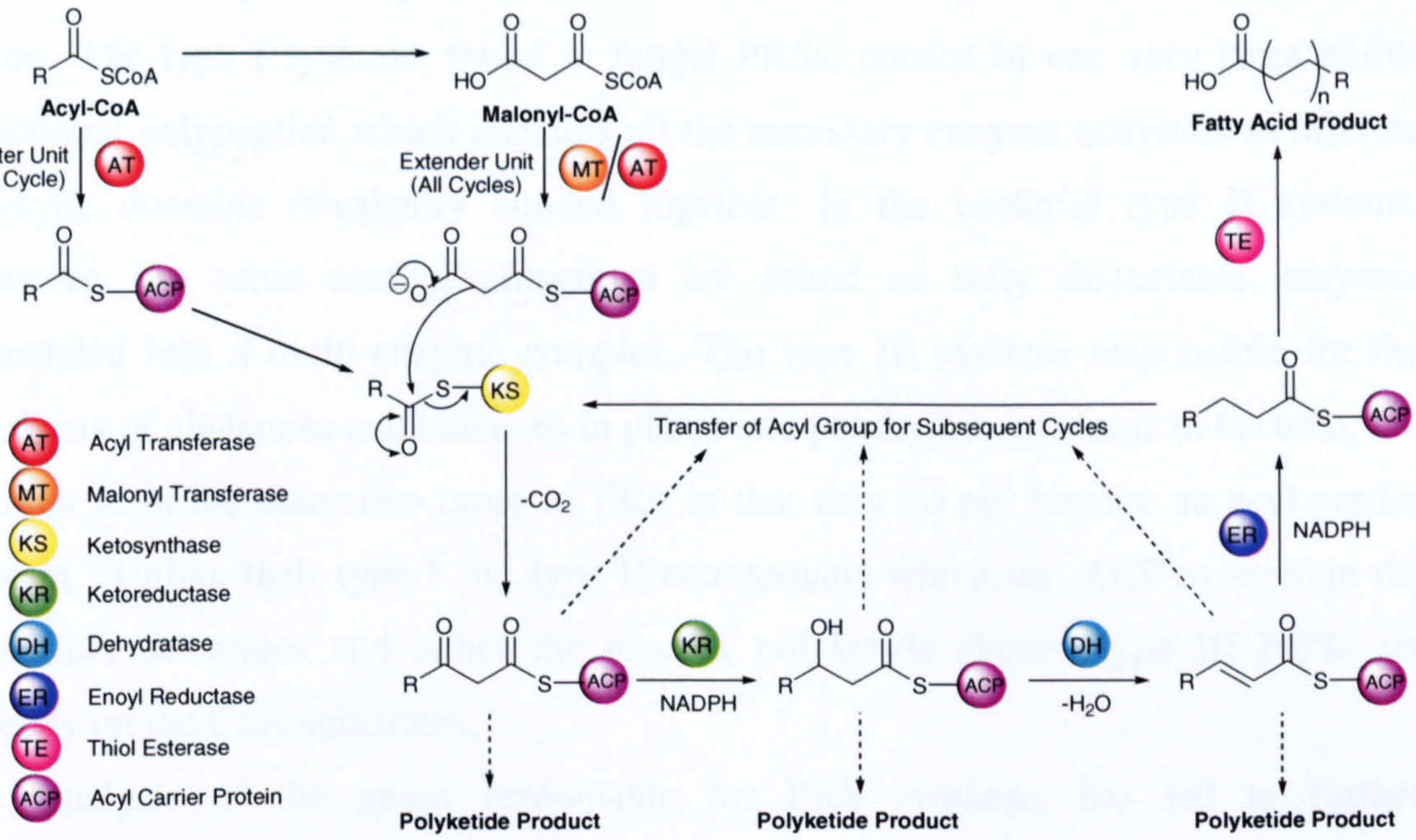


Figure 1: Examples of polyketide secondary metabolites.

Polyketides are assembled by multifunctional enzyme complexes called polyketide synthases (PKSs) in a manner analogous to fatty acid biosynthesis. The PKS contains all the catalytic domains required to complete the synthesis, in tandem with an acyl carrier protein (ACP) which functions as a transport system to deliver the chain to the various enzymes as required.

Firstly, a starter unit is loaded onto the acyl transferase domain of the PKS and is transferred onto the ketosynthase (Scheme 1). A malonyl extender unit is then loaded onto the malonyl transferase domain *via* malonyl-CoA (itself derived from acetyl-CoA). These two units undergo a condensation reaction catalysed by the ketosynthase to form acetoacetate. This is then passed on to the ketoreductase (which reduces the keto group), dehydratase (which removes water) and enoyl reductase (which reduces the double bond) as required. The nascent chain is then either transferred back onto the ketosynthase domain, where it accepts another extender unit and starts off another

cycle, or it is passed onto the thiol esterase which cleaves the chain from the PKS and releases it, usually as the free acid or as an acyl ester.



Scheme 1: Biosynthesis of fatty acid and polyketide metabolites.

Whereas the fatty acid synthases (FASs) usually have set starter and extender units (acetyl-CoA and malonyl-CoA respectively) and each successive chain elongation step is followed by a fixed sequence of ketoreduction, dehydration and enoyl reduction, the PKSs have been shown to accept a variety of starter and extender units (Figure 2) and to undergo some, all or none of the functional group modifications at each cycle, resulting in more structurally varied products.

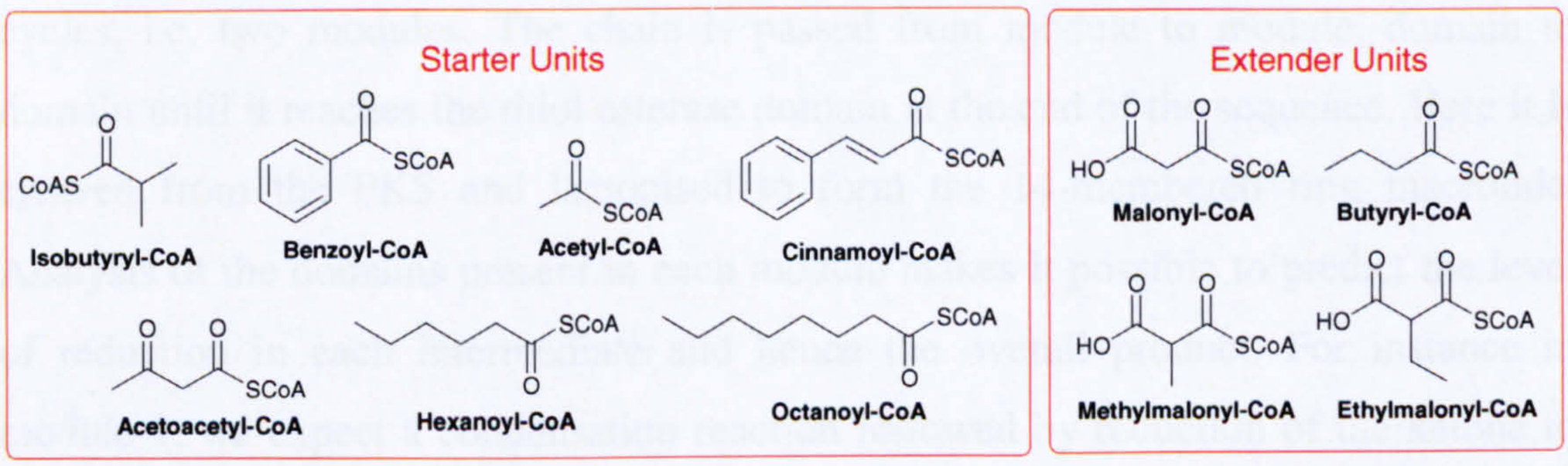


Figure 2: Examples of starter and extender units accepted by the polyketide synthases.

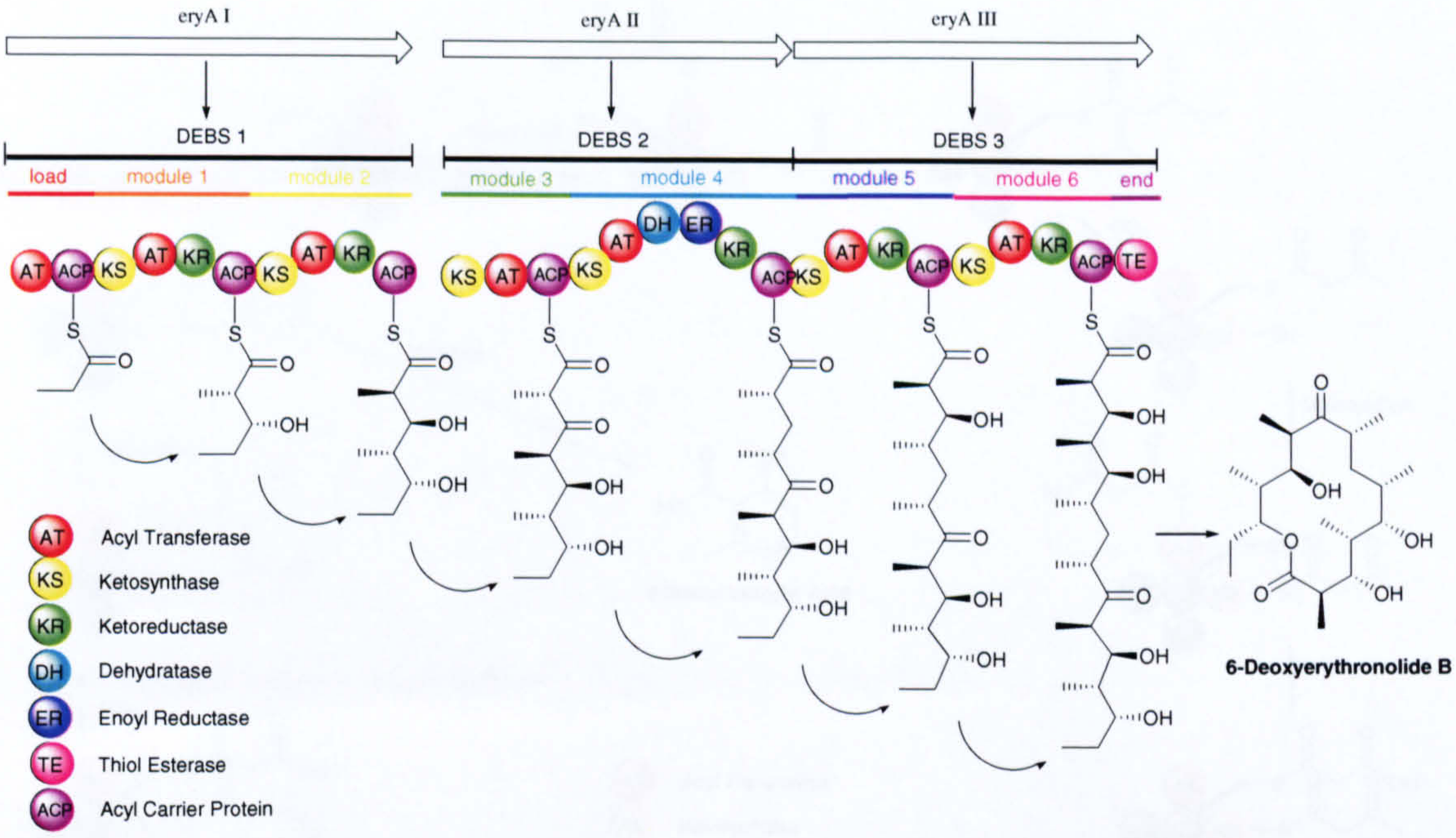
Further structural alterations can also be applied to the basic polyketide structure after assembly by tailoring enzymes. Examples of such modifications include

methylation (catalysed by *S*-adenosyl methionine (SAM)) and cyclisation (aided by the cyclases).

To date, polyketide synthases have been found to adopt three different structural forms. The type I systems, found in fungal PKSs, consist of one very large multi-functional polypeptide which contains all the necessary enzyme activities as discrete catalytic domains covalently bonded together. In the bacterial type II systems, however, the same catalytic functions are found as fully dissociable enzymes associated into a multi-enzyme complex. The type III systems responsible for the synthesis of chalcones and stilbenes in plants and polyhydroxyphenols in bacteria, are distinct from the other two types of PKS in that they do not involve an acyl carrier protein.[4] Unlike their type I and type II counterparts which use ACP to activate the acyl-CoA substrates and tether the nascent polyketide chains, type III PKSs act directly on the CoA substrates.

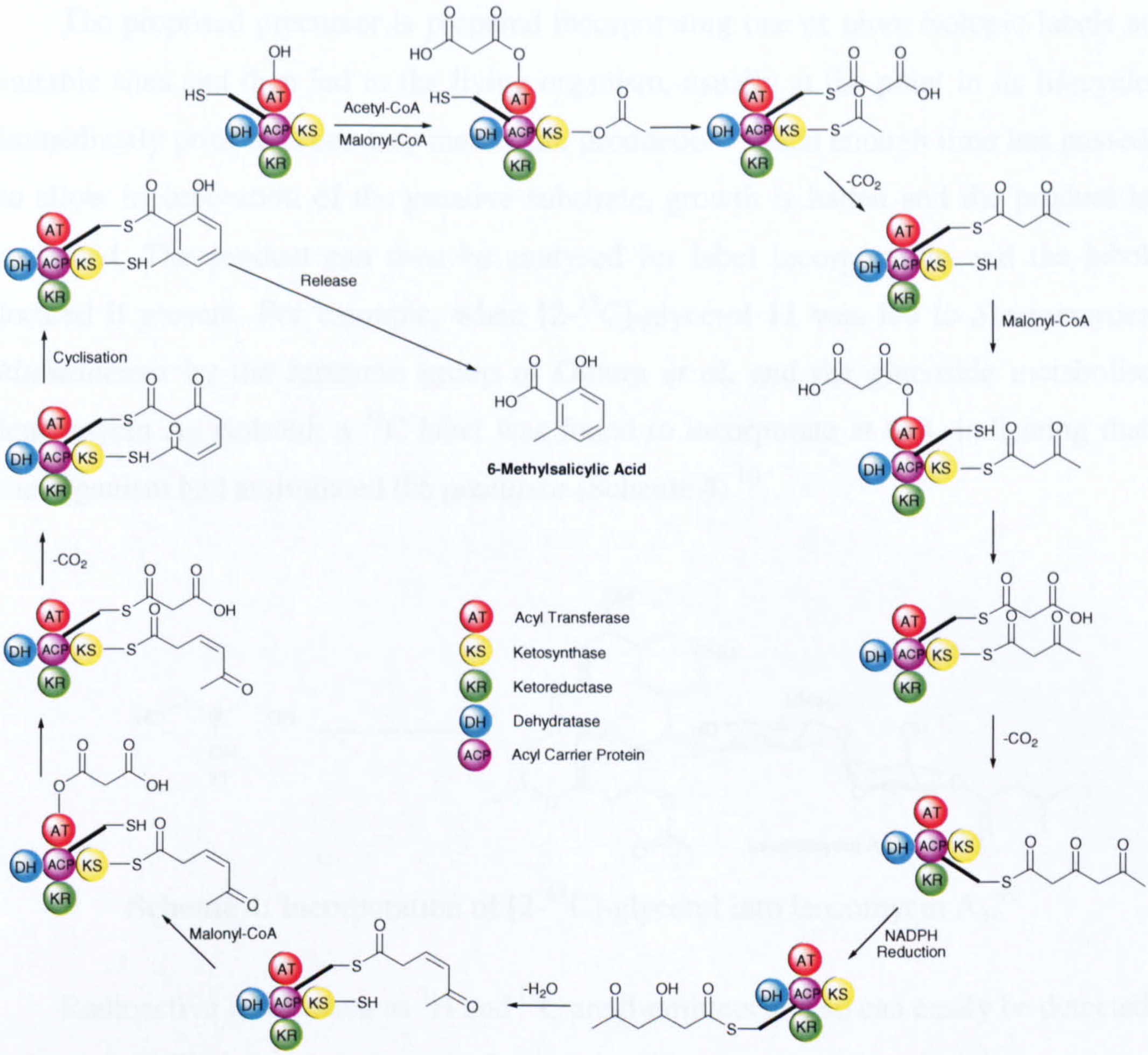
Analysis of the genes responsible for PKS synthesis has led to further characterisation. It was established that systems could be either modular or iterative. Modular PKSs contain a separate set of enzymes for each catalytic cycle. An example of this type of PKS is that responsible for the synthesis of 6-deoxyerythronolide B (a precursor to erythromycin) in the bacterium *Saccharopolyspora erythraea*.[5] It was found that the genes responsible for 6-deoxyerythronolide B, were organised into three large open reading frames (ORFs) designated *eryAI*, *eryAII* and *eryAIII* respectively, which encode for three large multifunctional proteins (~350 kDa each), entitled DEBS 1, DEBS 2 and DEBS 3 (Scheme 2).

Each of these proteins contains the catalytic domains for two condensation cycles, i.e. two modules. The chain is passed from module to module, domain to domain until it reaches the thiol esterase domain at the end of the sequence. Here it is cleaved from the PKS and lactonised to form the 14-membered ring macrolide. Analysis of the domains present in each module makes it possible to predict the level of reduction in each intermediate and hence the overall product. For instance in module 1, we expect a condensation reaction followed by reduction of the ketone to an alcohol, whereas in module 4 a condensation reaction followed by a complete round of reduction is expected.



Scheme 2: Domain organisation of the 6-deoxyerythronolide B polyketide synthase.[5]

Iterative PKSs on the other hand, use the same constituent enzymes repeatedly as necessary. Each cycle does not necessarily use each catalytic domain, so again, there are many possible structural variants. An example of this type of PKS is that responsible for 6-methylsalicylic acid.[6] Comprising of only 5 domains (ketosynthase, acyl transferase, dehydratase, ketoreductase and acyl carrier protein respectively), it catalyses three rounds of chain extension, carrying out different levels of reductive processing at each stage (Scheme 3). For example, after the first condensation no reduction whatsoever occurs whereas after the second the ketoreductase and dehydratase enzymes act to reduce the newly formed ketone to an olefin. During the third cycle, the chain undergoes cyclisation, dehydration and enolisation to afford the acid product. There is much speculation as to how this specificity arises.



Scheme 3: Proposed biosynthetic pathway for 6-methylsalicylic acid.[6]

1.2 Elucidation of Polyketide Biosynthetic Pathways

Many approaches are used to study biosynthetic pathways and to substantiate true biosynthetic intermediates and precursors. These include:

(i) employing isotopically-labelled compounds;

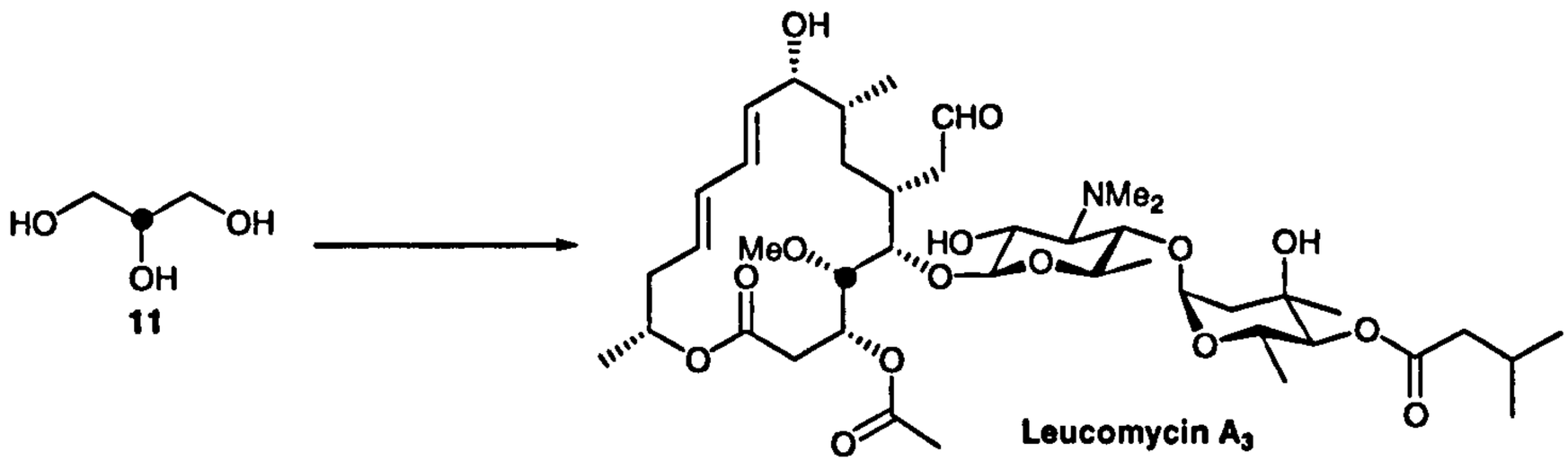
(ii) studying extracted and purified enzyme systems *in vitro*;

(iii) investigating the effects of organisms with blocked biosynthetic pathways.

1.2.1 Isotopic Labels

Isotopic labels have been used for decades in the elucidation of biosynthetic pathways. They can reveal a great deal about possible precursors, intermediates, mechanisms, reaction sites, tautomerism, migrations, enzyme specificity, the order of reaction etc., and so are widely utilised.[7,8,9]

The proposed precursor is prepared incorporating one or more isotopic labels at suitable sites and then fed to the living organism, usually at the point in its lifecycle immediately prior to secondary metabolite production. When enough time has passed to allow incorporation of the putative substrate, growth is halted and the product is extracted. The product can then be analysed for label incorporation and the label located if present. For example, when [2-^{13}C]-glycerol **11** was fed to *Streptomyces kitasatoensis* by the Japanese group of Omura *et al.* and the glucoside metabolite leucomycin A_3 isolated, a ^{13}C label was found to incorporate at C-4, indicating that the organism had assimilated the precursor (Scheme 4).[10]

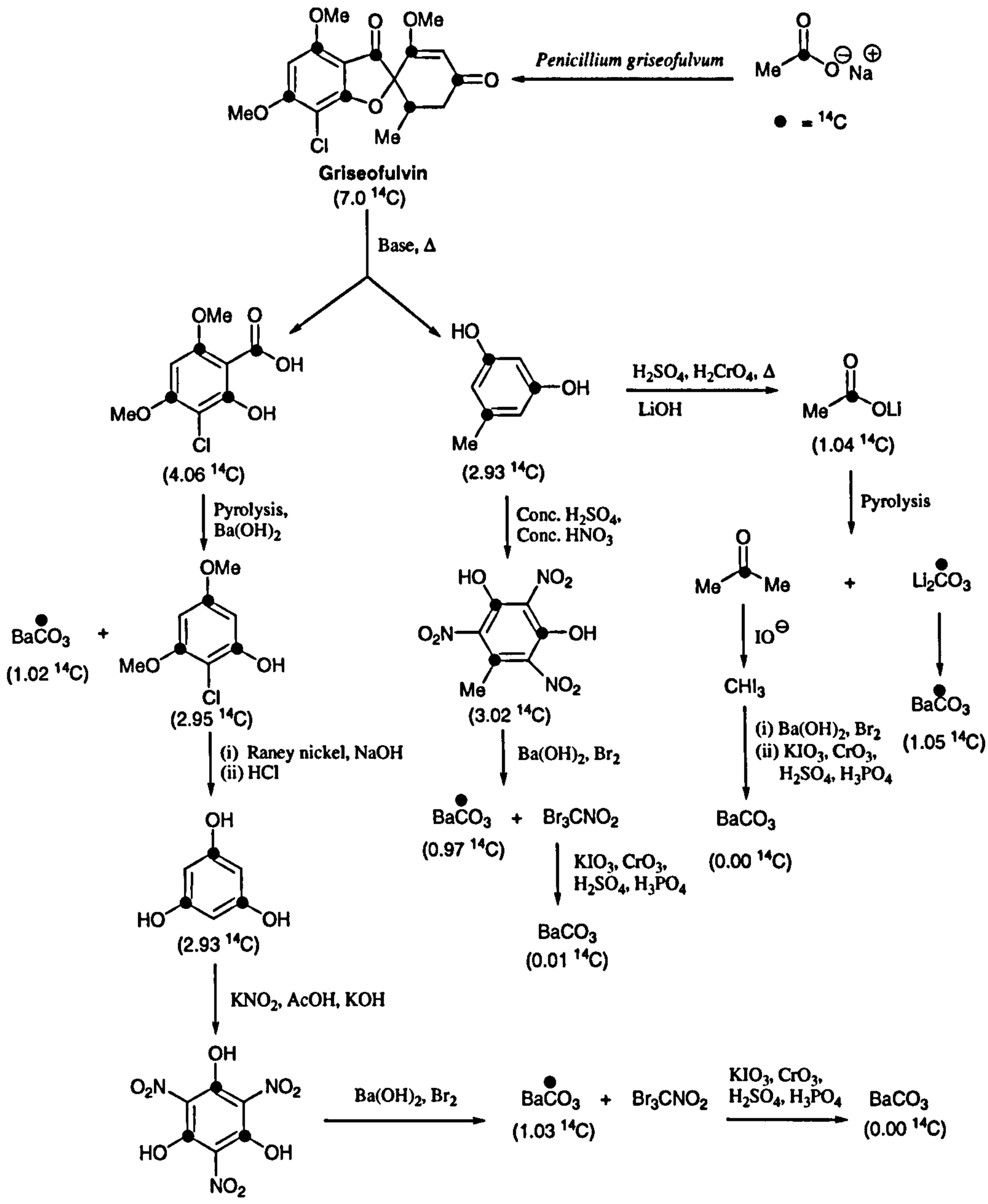


Scheme 4: Incorporation of [2-^{13}C]-glycerol into leucomycin A_3.[10]

Radioactive labels such as ^{3}H and ^{14}C are β-emitters and so can easily be detected using a scintillation counter to test for activity. However, locating the exact position of the label in the target molecule can usually only be achieved by degradation studies (the exception being tritium, which is NMR active[7]). This involves progressively breaking the molecule down into smaller known components and measuring the radioactivity at each stage, as for instance in the work of Birch *et al.* on griseofulvin[11] (Scheme 5). This can be tedious and very time consuming and so radioactive labels are no longer used to a great extent in microbial systems, although they can be useful tracers.

Stable isotopic labels on the other hand, can be detected by NMR spectroscopy and mass spectrometry. This makes locating the incorporated label much easier than for radiolabels. Although direct NMR analysis is restricted to nuclei which possess a nuclear magnetic moment (e.g. ^{1}H, ^{13}C and ^{15}N), various techniques have been employed to indirectly ascertain the position of isotopes, such as ^{18}O, which are not NMR active.[7] The first step is always to completely assign the natural abundance spectra. These act as reference spectra to which the spectra obtained post-feeding can be compared.

Deuterium is a commonly used isotopic label. It has a low natural abundance of 0.16% and can be detected directly by ^{2}H NMR spectroscopy as it has a nuclear spin, I = 1. However, as deuterium has a small gyromagnetic ratio ^{2}H NMR spectroscopy is not very sensitive and due to quadrupolar relaxation of the nucleus, signals are broad leading to poorly resolved spectra.[9] Thus, a complementary method of detection is to look for the disappearance (or a reduction in the integral), of a signal in the ^{1}H NMR spectrum. By comparison with the natural abundance spectrum the site of incorporation can be determined.



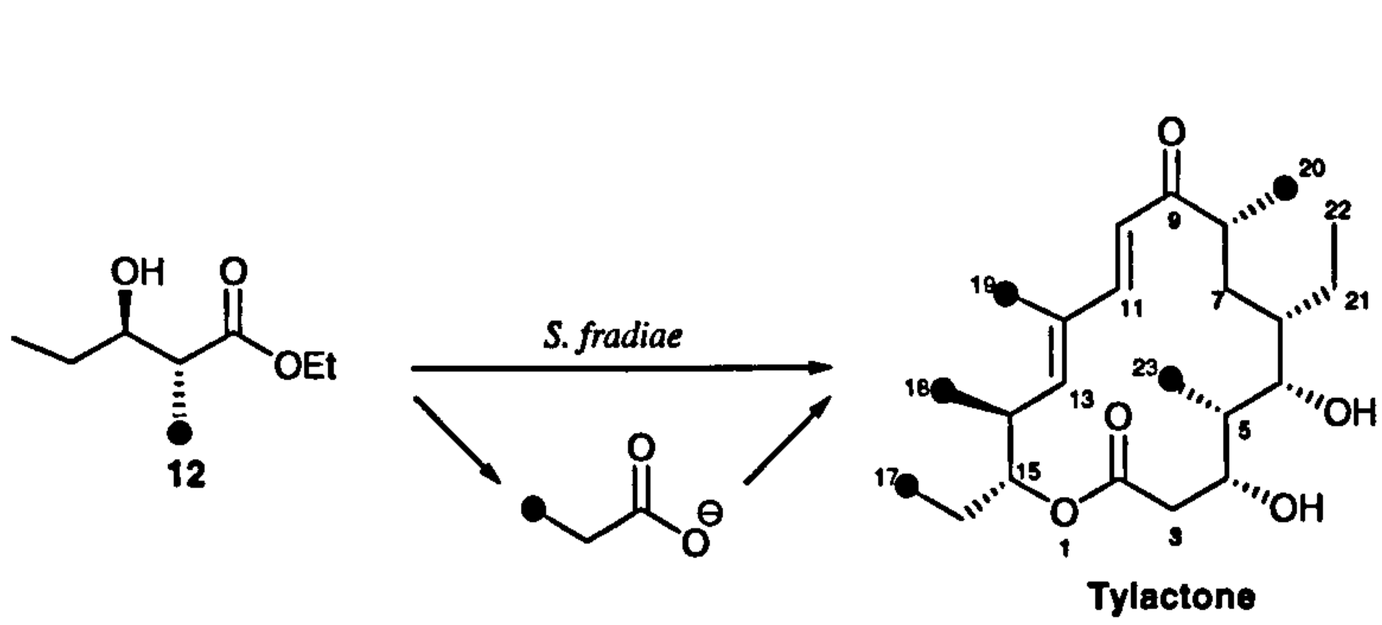
Scheme 5: Degradation studies carried out by Birch *et al.* to locate the positions of incorporation of ^{14}C in griseofulvin.[11]

Carbon-13 is another commonly used isotopic label. It has a natural abundance of approximately 1.1%.[7] The site of incorporation is easily determined by an enhanced signal in the ^{13}C NMR spectrum.[8] ^{1}H-^{13}C coupling may also be observed in the ^{1}H NMR spectrum.[7,8]

Multiple labels allow coupling patterns to be exploited. For instance, spin-spin coupling between two vicinal ^{13}C labels produces a doublet (J between 30-90 Hz)[8] in the ^{13}C spectrum. This means that even very low levels of incorporation (<1%) can be detected as satellite signals either side of the natural abundance peak, making this a very sensitive technique.

Although not itself NMR active, ^{18}O can be detected indirectly from the ^{13}C NMR spectrum by α-isotope effects.[9] The ^{13}C-^{18}O signal appears 0.01-0.035 ppm upfield of the corresponding ^{13}C-^{16}O signal and the ^{13}C=^{18}O signal appears 0.03-0.5 ppm upfield of the corresponding ^{13}C=^{16}O signal.

Incorporation of more than one isotopic label can also be useful in determining whether a precursor is incorporated intact into a product. For example, in the case of a precursor incorporating vicinal ^{13}C labels, a loss of ^{13}C-^{13}C coupling indicates that the mechanism by which the final compound is formed involves cleavage of that bond.[8] It is also possible that the precursor may have been metabolised previously and broken down by the organism prior to incorporation, usually indicated by multiple ^{13}C incorporation. Hutchinson and co-workers encountered degradation problems of this nature whilst attempting to ascertain the biosynthetic pathway of tylactone.[12] Having fed ethyl ester **12** to *Streptomyces fradiae*, the group were surprised to discover that C-18 of tylactone was not selectively ^{13}C enriched (Scheme 6). Instead, it was equally enriched at C-17, C-18, C-19, C-20 and C-23 (all known to derive from C-3 of propionate), indicating that the precursor had degraded to propionate prior to incorporation.



Scheme 6: Multiple sites of ^{13}C incorporation in tylactone indicating prior degradation of precursor.[12]

Incorporation of one or more deuterium atoms either one (α) or two (β) bonds away from a ^{13}C nucleus also causes diagnostic chemical shifts and coupling patterns.[9] In molecules with one deuterium vicinal to the ^{13}C, the ^{13}C signal is α-shifted 0.3 ppm upfield in the ^{13}C NMR spectrum and appears as a 1:1:1 triplet due to ^{2}H-^{13}C coupling. For each additional deuterium there is a further upfield shift and a further increase in multiplicity (Figure 3). β-isotope shifts, on the other hand, are typically in the region of 0.01 to 0.1 ppm and appear as singlets due to the minute ^{2}H-^{13}C coupling values.

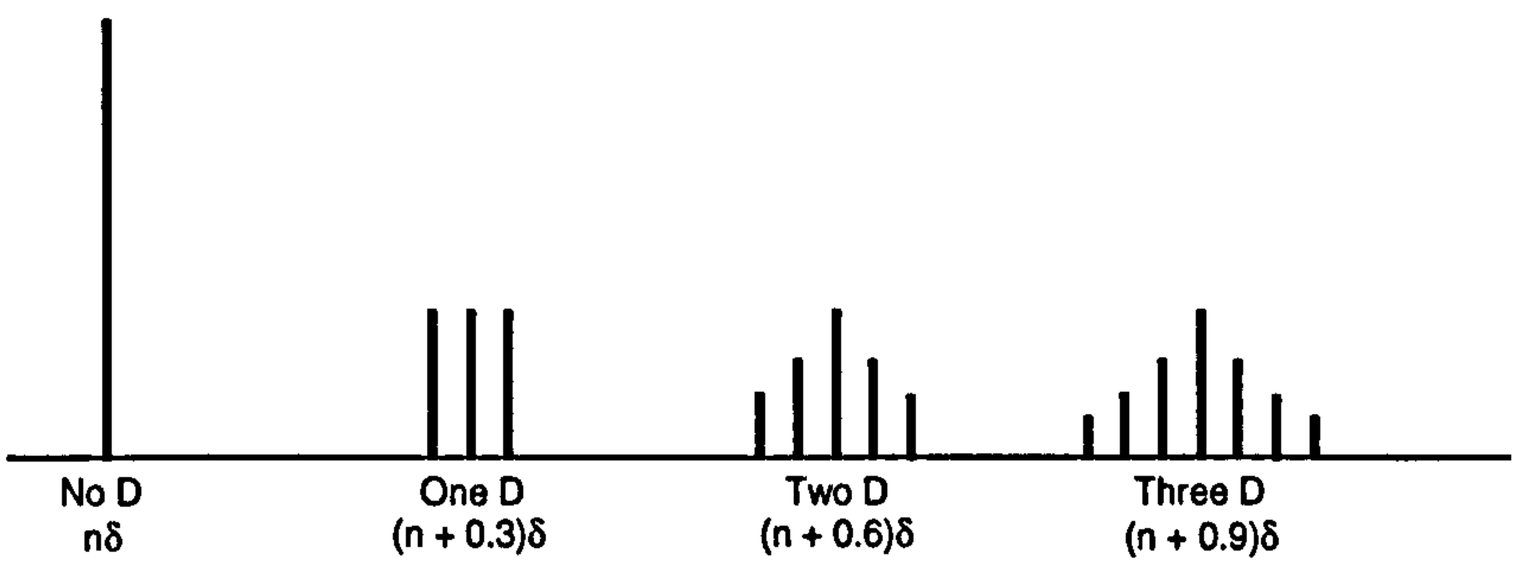
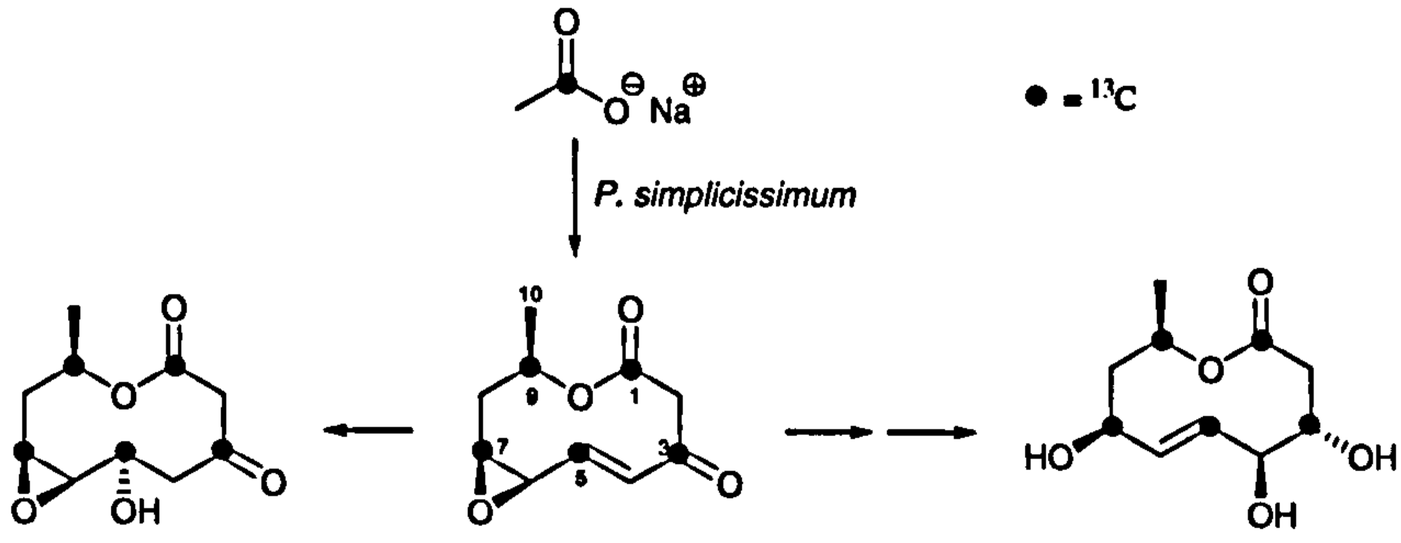


Figure 3: Hypothetical α-isotope effects observed in the ^{13}C NMR spectrum due to ^{2}H-^{13}C coupling.

The relative efficiency of incorporation of labelled precursors is another source of information. In theory, the greater the level of incorporation, the less metabolism that precursor has undergone. Mayer and Thiericke used this hypothesis to good effect in their study of the decarestrictines.[13] Having fed sodium [1-$^{13}C_2$]-acetate to the fungus *Penicillium simplicissimum* and isolated decarestrictines B, D and F, the relative enrichment levels for C-1, C-3, C-5, C-7 and C-9 were found to be consistently much higher for decarestricine F (Scheme 7). They therefore concluded that decarestrictine F is produced earlier in the biosynthetic pathway than B and D. They could also deduce the order in which the acetate units were combined in this family of metabolites, starting with the C-9–C-10 unit and working anti-clockwise around the molecule.



Carbon	Specific Incorporation
C-1	1.6
C-3	3.6
C-5	5.5
C-7	11.0
C-9	7.9

Carbon	Specific Incorporation
C-1	5.9
C-3	7.6
C-5	19.6
C-7	37.5
C-9	18.3

Carbon	Specific Incorporation
C-1	1.1
C-3	1.6
C-5	1.3
C-7	1.2
C-9	1.2

Scheme 7: Proposed biosynthetic relationship of decarestrictine B, D and F.[13]

However, while the relative rates of incorporation are indicative of where a particular intermediate lies in the biosynthetic pathway, there are many other factors to consider as to why a particular incorporation is high or low (such as ease of penetration of the precursor, competing mechanisms, culture conditions etc.) and so they do not conclusively prove the order in which biosynthetic intermediates are formed.

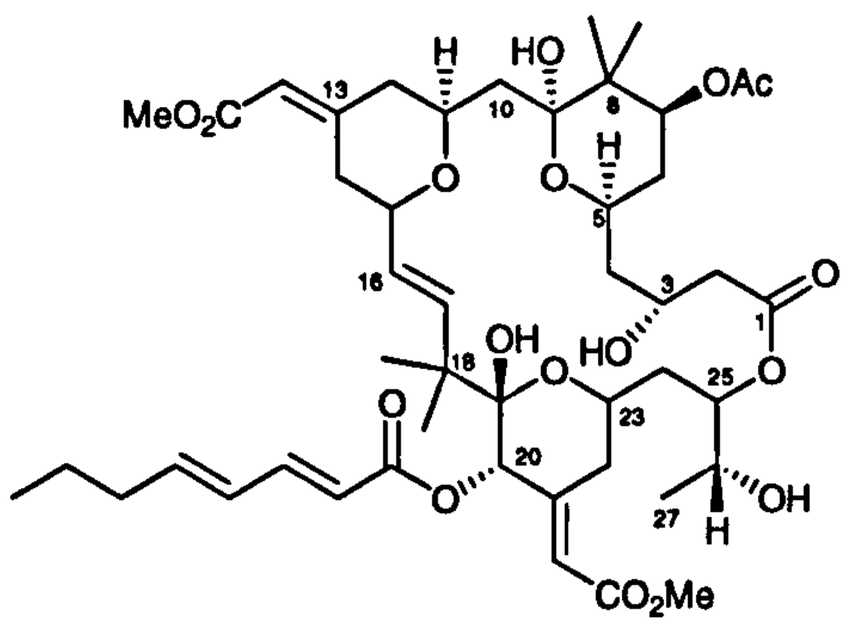
1.2.2 Cell-Free Enzyme Systems

Cell-free enzyme systems allow investigation of enzyme function, substrate specificity, intermediates, stereoselectivity, possible inhibitors, pH/temperature dependence etc. in a controlled *in vitro* environment. They are obtained by lysis of the cell membranes and extraction of the enzymes within. A variety of methods have been employed, including digestion with enzymes such as lysozyme or lysostaphin, chemical degradation using alkali or detergent and physical destruction of the cell wall by sonication, osmotic shock, shearing or grinding with abrasives (such as glass beads, sand).[14,15] Unfortunately, due to the harsh conditions employed and the sensitivity of many enzyme complexes, these techniques are not amenable to all systems. Often enzymes become denatured when removed from their natural environment and lose bioactivity.

Cell-free extracts are of particular benefit in complex systems - such as plant and mammalian systems - where incorporation of labelled compounds is so diluted by the metabolic processes that it is difficult to detect the labels after isolation, in systems where only minute quantities of the compound under investigation can be detected in

the crude extract or in situations where the putative precursor is unable to penetrate to the site of synthesis due to permeability or transport problems.

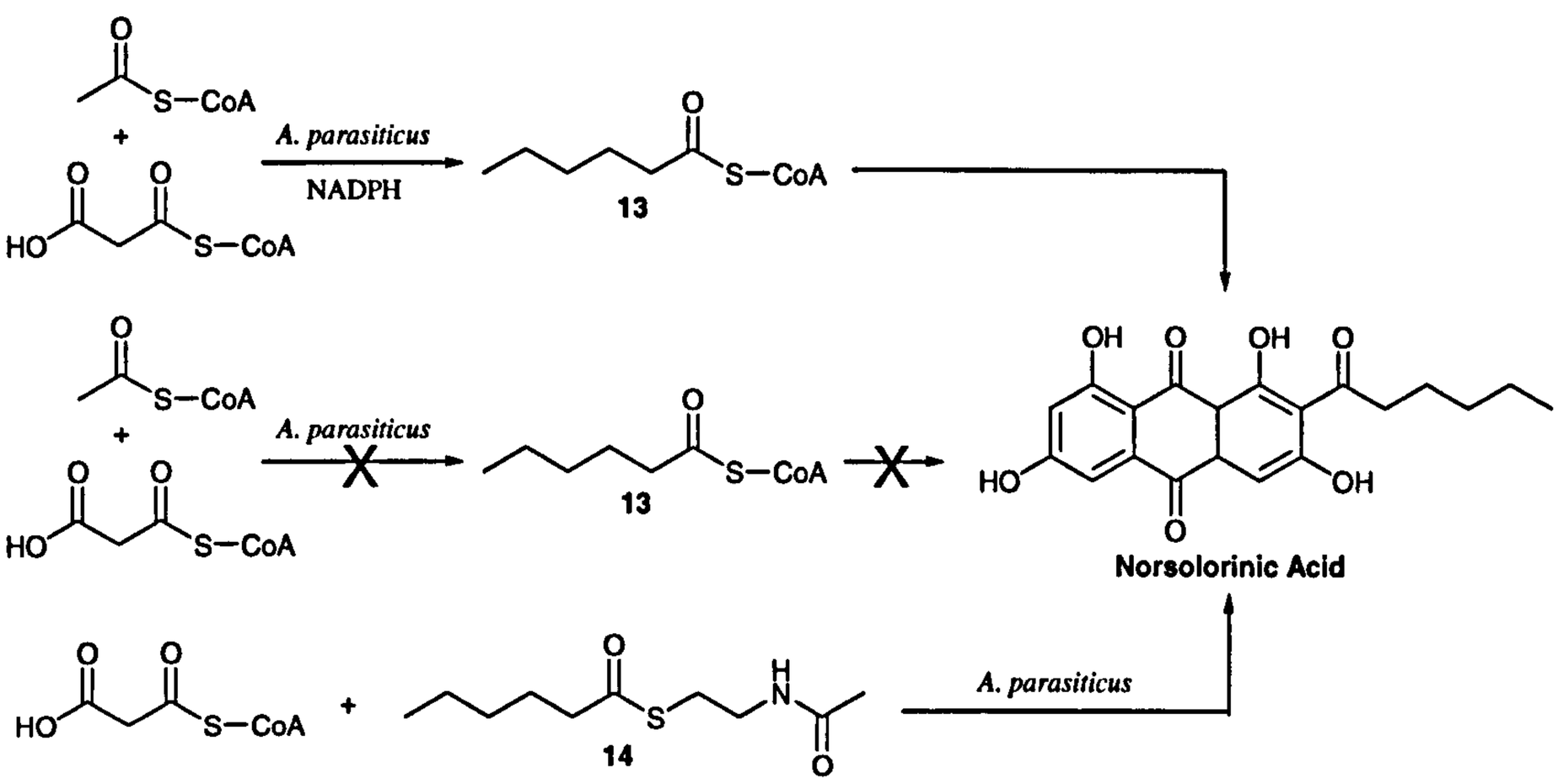
Bryostatin 1, present in trace quantities in the marine bryozoan *Bugula neritina*, exhibits selective activity against B-cell lymphomas and leukaemia.[16] Kerr *et al.* used cell-free extracts of *B. neritina* to investigate the biosynthetic precursors of this valuable metabolite.[17] They were particularly interested in the origins of the gem dimethyl groups at C-8 and C-18 (which they proposed could be derived from SAM or isobutyrate), the ethylidene substituents at C-13 and C-21 (which they suggested were either the result of direct acetate addition to the polyketide chain or an intact four carbon unit) and the C-25–C-27 starter unit (possible sources included propionate, glycerol and *n*-butyrate). The group administered radiolabelled precursors to the extracts and measured the activity of the resulting bryostatin. [^{3}H]-Acetate, [^{3}H]-SAM and [^{3}H]-glycerol all showed incorporation (though the sites of incorporation were not determined) whereas [^{14}C]-propionate, [^{14}C]-*n*-butyrate, [^{14}C]-isobutyrate and [^{14}C]-succinate failed to incorporate. The PKS proposed to be responsible for bryostatin biosynthesis was later found by Haygood and co-workers in the genome of the symbiotic bacterium "*Candidatus* Endobugula sertula".[18] This was found to contain methyl transerase domains (known to use SAM as a methyl source) verifying at least one of these results. Haygood and co-workers are also of the opinion that acetyl-CoA condensation onto the β-keto groups at C-13 and C-21 by *BryR* during biosynthesis is the most likely origin of the ethylidene moieties.



Bryostatin 1

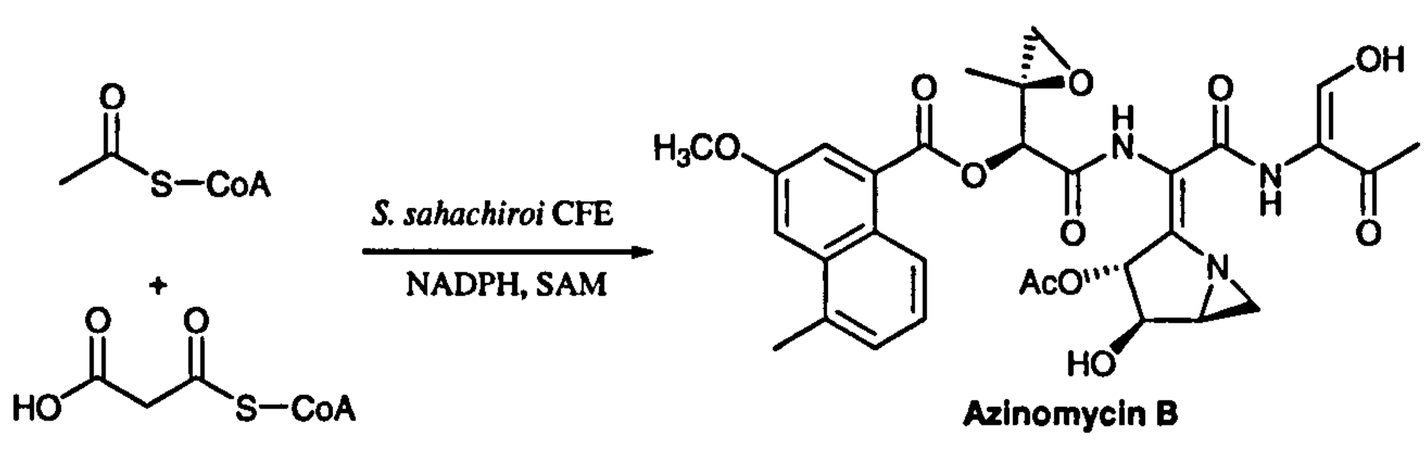
Unlabelled substrates can also be used for feeding studies with cell-free extracts. Because there is little or no background production, any biosynthesis of the desired metabolite can be taken as a positive incorporation. For example, Watanabe and Townsend[19] extracted and partially purified the PKS responsible for norsolorinic acid

(the first known intermediate in the biosynthetic pathway to the carcinogen aflatoxin B_1) from *Aspergillus parasiticus*. In the presence of acetyl-CoA, malonyl-CoA and NADPH, norsolorinic acid is produced by the extracts *via* hexanoate **13** (Scheme 8). In the absence of NADPH, however, the hexanoate intermediate **13** cannot be formed and thus no norsolorinic acid is observed. Production of the acid is restored when exogenous hexanoyl thiol ester **14** is fed to the system, thus confirming its intermediacy in the biosynthetic pathway.



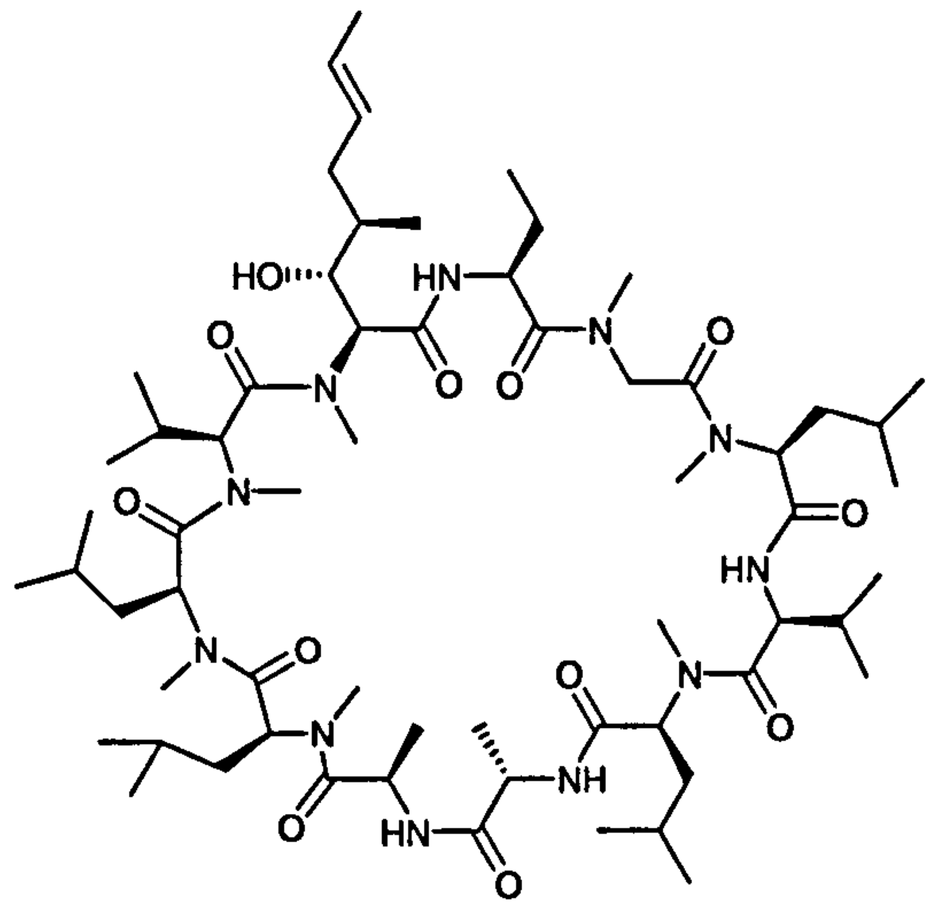
Scheme 8: Biosynthesis of norsolinic acid by cell-free extracts of *A. parasiticus*.[19]

Watanabe and co-workers used cell-free extracts to probe the cofactor requirements for the biosynthesis of the antitumor agent azinomycin B in *Streptomyces sahachiroi*.[20] NADPH was found to be essential for azinomycin B production, as was SAM. Flavin adenine dinucleotide (FAD) and tetrahydrofolate on the other hand were not required and rather surprisingly for a proposed polyketide, adenosine triphosphate (ATP) did not appear to have much of an effect on azinomycin B production.

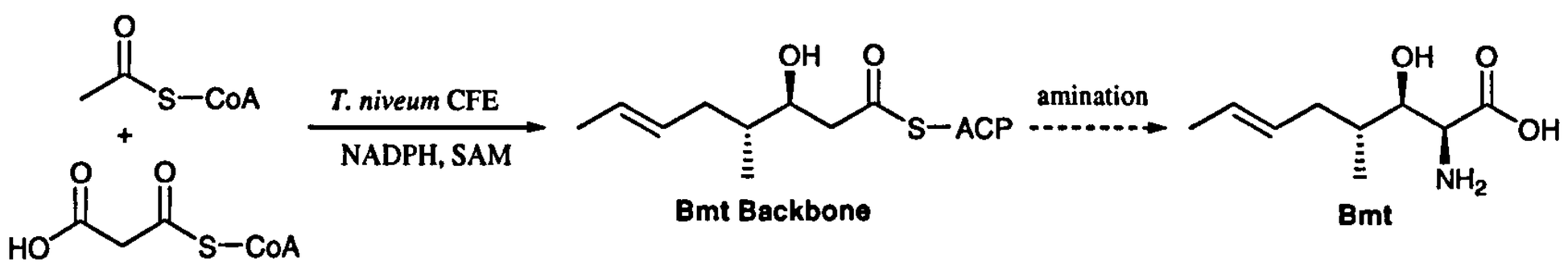


Scheme 9: Cofactor requirements for azinomycin B production in cell-free extracts of *S. sahachiroi*.[20]

The immunosuppresent cyclosporin A, produced by the fungus *Tolypocladium niveum*, is comprised of eleven constituent amino acids.[21] The biosynthesis of one of these, designated Bmt, is believed to take place in two phases; a polyketide back bone assembly followed by introduction of the amino group. In order to investigate the first phase of Bmt biosythesis, Schneider-Scherzer and co-workers prepared and partially purified cell-free extracts from *T. niveum*.[22] By assaying fractions for NADPH consumption dependent on acetyl-CoA, malonyl-CoA and SAM they were able to isolate the polyketide synthase responsible for the Bmt backbone. The PKS could now be investigated in isolation and its polyketide product, designated Bmt backbone, and substrate specificity determined.



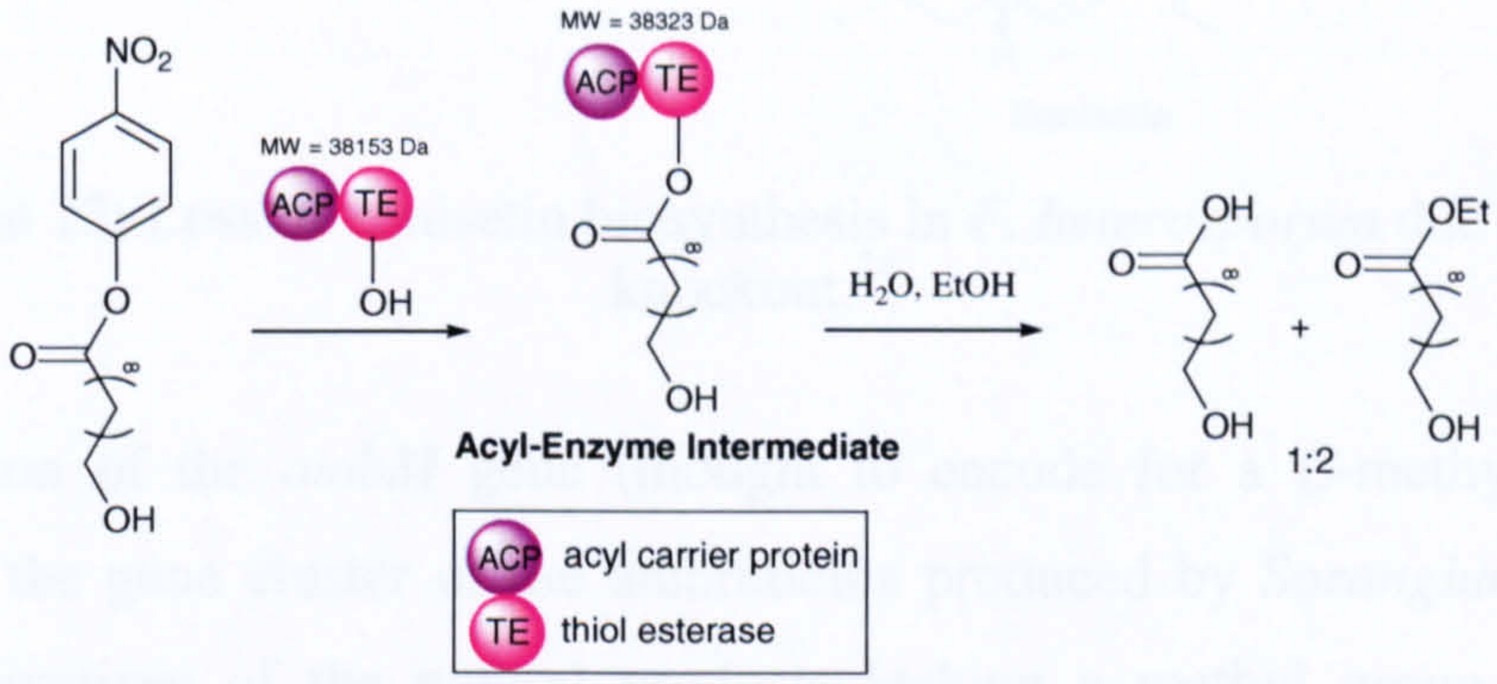
Cyclosporin A



Scheme 10: Investigation of Bmt biosynthesis in cell-free extracts of *T. niveum*.[22]

Staunton and co-workers used cell-free extracts to good effect in determining the mode of action and substrate specificity of the thiol esterase domain of the 6-deoxyerythronolide B PKS.[23] The enzyme was found to form acyl-enzyme intermediates which then underwent nucleophilic displacement (by either an external nucleophile such as water or alcohol or an internal hydroxyl group) to release the chain from the enzyme (Scheme 11). The enzyme was found to have broad substrate

specificity, accepting a variety *p*-nitrophenyl esters and thiol esters with varying acyl chain lengths and substitution patterns.



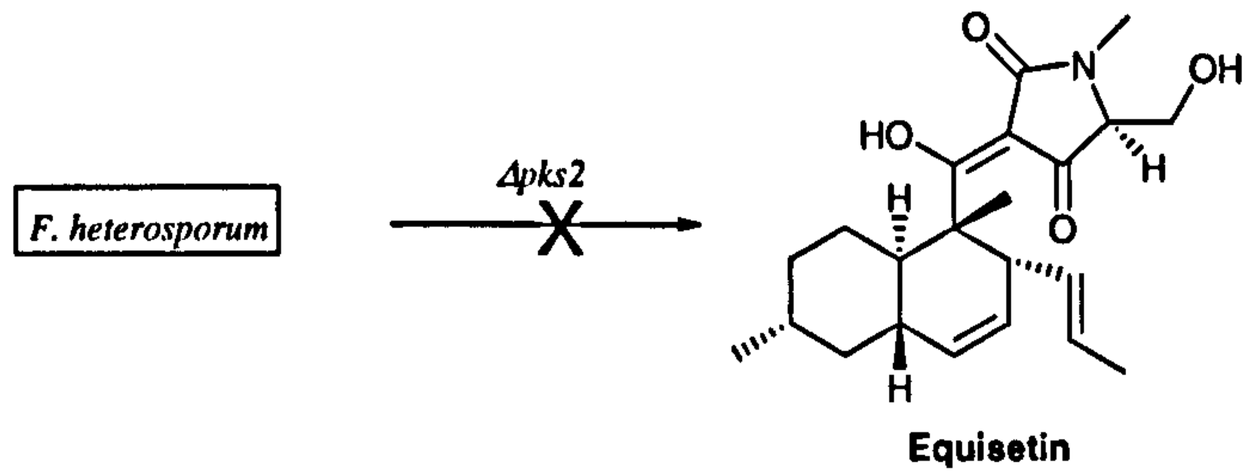
Scheme 11: Mode of action of thiol esterase domain of 6-deoxyerythronolide B.[23]

However, although cell-free extracts allow very specific experiments to be designed (especially in conjunction with modern genetic techniques) within a highly controlled environment and are generally good models, care must be taken in drawing parallels to more complex *in vivo* systems.

1.2.3 Organisms with Blocked Biosynthetic Pathways

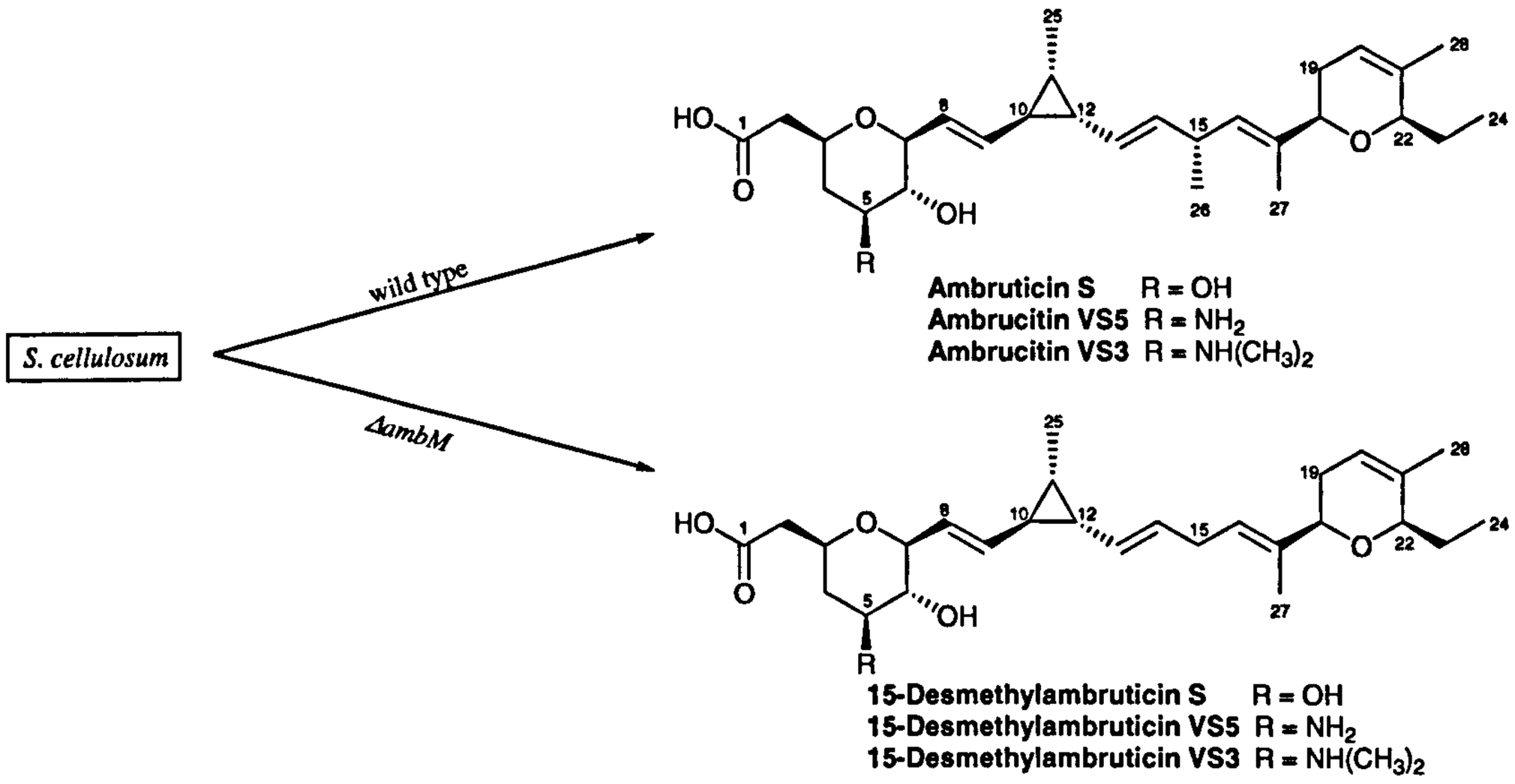
Introducing a block into an organism's biosynthetic pathway and analysing the results, is a valuable method of studying biosynthesis. The block may be endogenous, caused by a genetic mutation or it may be exogenous, induced by a chemical inhibitor.

In many cases a genetic mutation is used to determine the function of a particular gene or the cluster to which it belongs. For example, Schmidt and co-workers in searching for the genes responsible for equisetin synthesis, identified three polyketide synthase sequences within the *Fusarium heterosporum* genome, each a potential target.[24] In order to determine which of the three produced the polyketide, they performed a knockout mutagenesis on their preferred candidate, designated *pks2*, which resulted in a loss of equisetin production (Scheme 12). They concluded that *pks2* was indeed responsible for equisetin biosynthesis.



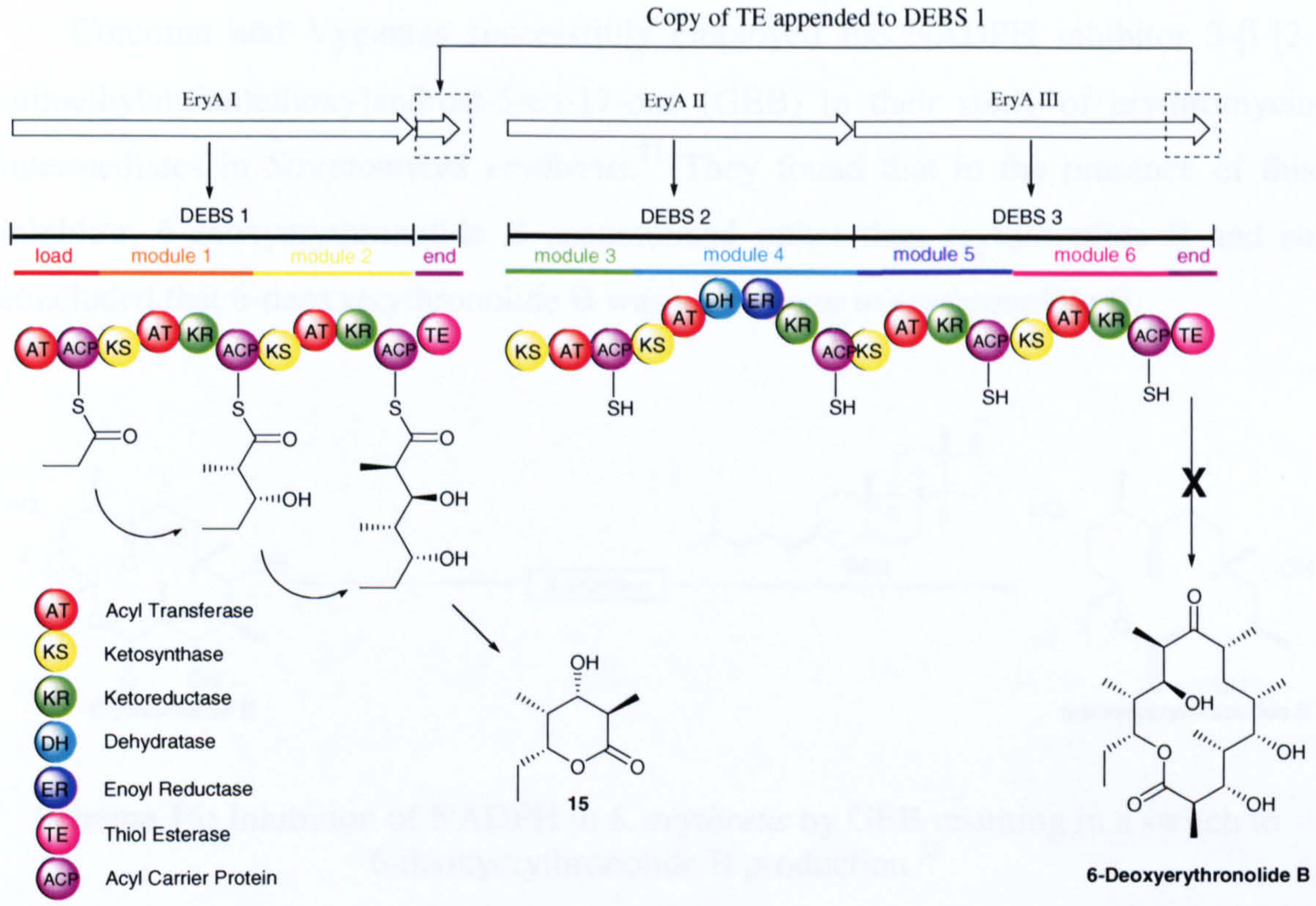
Scheme 12: Loss of equisetin biosynthesis in *F. heterosporum* due to *pks2* knockout.[24]

Disruption of the *ambM* gene (thought to encode for a *C*-methyl transferase enzyme), in the gene cluster of the ambruticins produced by *Sorangium cellulosum* leads to derivatives of the natural products lacking a methyl group at the C-15 position.[25]



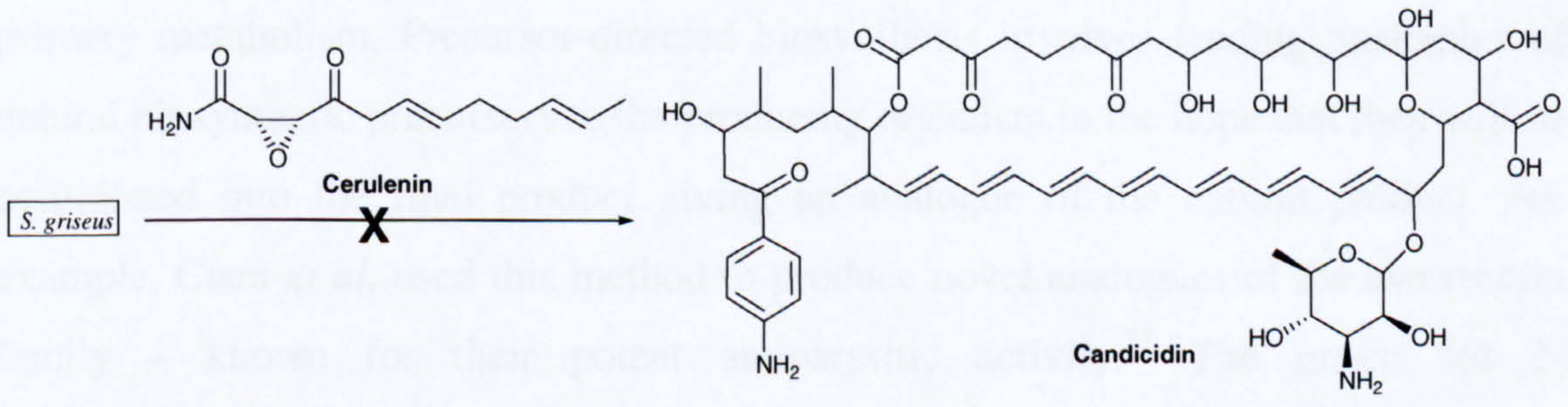
Scheme 13: Role of *ambM* in ambruticin biosynthesis ascertained by a knockout experiment.[25]

Leadlay, Staunton and co-workers also used an endogenous block to investigate the role of the DEBS 1 protein in 6-deoxyerythronolide B biosynthesis. By repositioning the thiol esterase domain from the end of DEBS 3 to the end of DEBS 1, they successfully interrupted the chain extension process and isolated triketide lactone **15**, the product of premature PKS release (Scheme 14).[26] Extending this methodology, two additional intermediates were obtained by relocating the thiol esterase to the end of modules 3 and 5.[27,28]



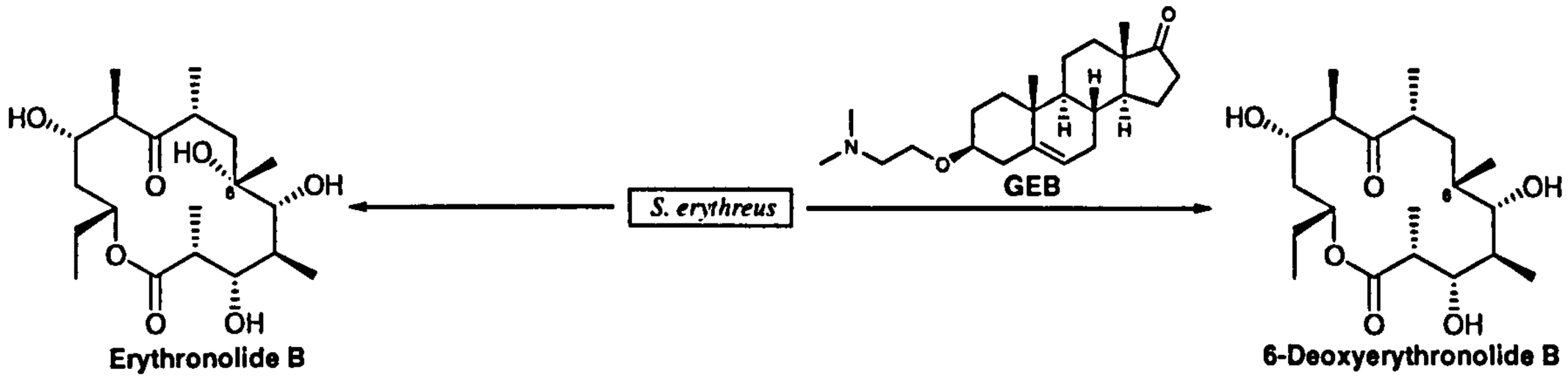
Scheme 14: Repositioning of the thiol esterase domain of 6-deoxyerythronolide B to the end of DEBS 1 to block further biosynthesis.[26]

Exogenous blocks can also yield valuable biosynthetic information. For instance, Martin and McDaniel specifically inhibited production of the antibiotic candicidin in *Streptomyces griseus* using cerulenin, an inhibitor known to block the condensation of malonyl-CoA subunits in the formation of fatty acids (Scheme 15).[29] As the synthesis of other proteins and RNA were not affected they concluded that candicidin must be prepared *via* the polyketide biosynthetic pathway (known to be highly analogous to the fatty acid biosynthetic pathway). Omura *et al.* also used this inhibitor to show that the macrolides antibiotic tylosin was polyketide derived.[30]



Scheme 15: Inhibition of candicidin production in *S. griseus* by cerulenin.[29]

Corcoran and Vygantas successfully employed the NADPH inhibitor 3-β-[2-(dimethylamino)ethoxy]androst-5-en-17-one (GEB) in their study of erythromycin intermediates in *Streptomyces erythreus*.[31] They found that in the presence of this inhibitor, 6-deoxyerythronolide B accumulated rather than erythronolide B and so concluded that 6-deoxyerythronolide B was a precursor to erythronolide B.



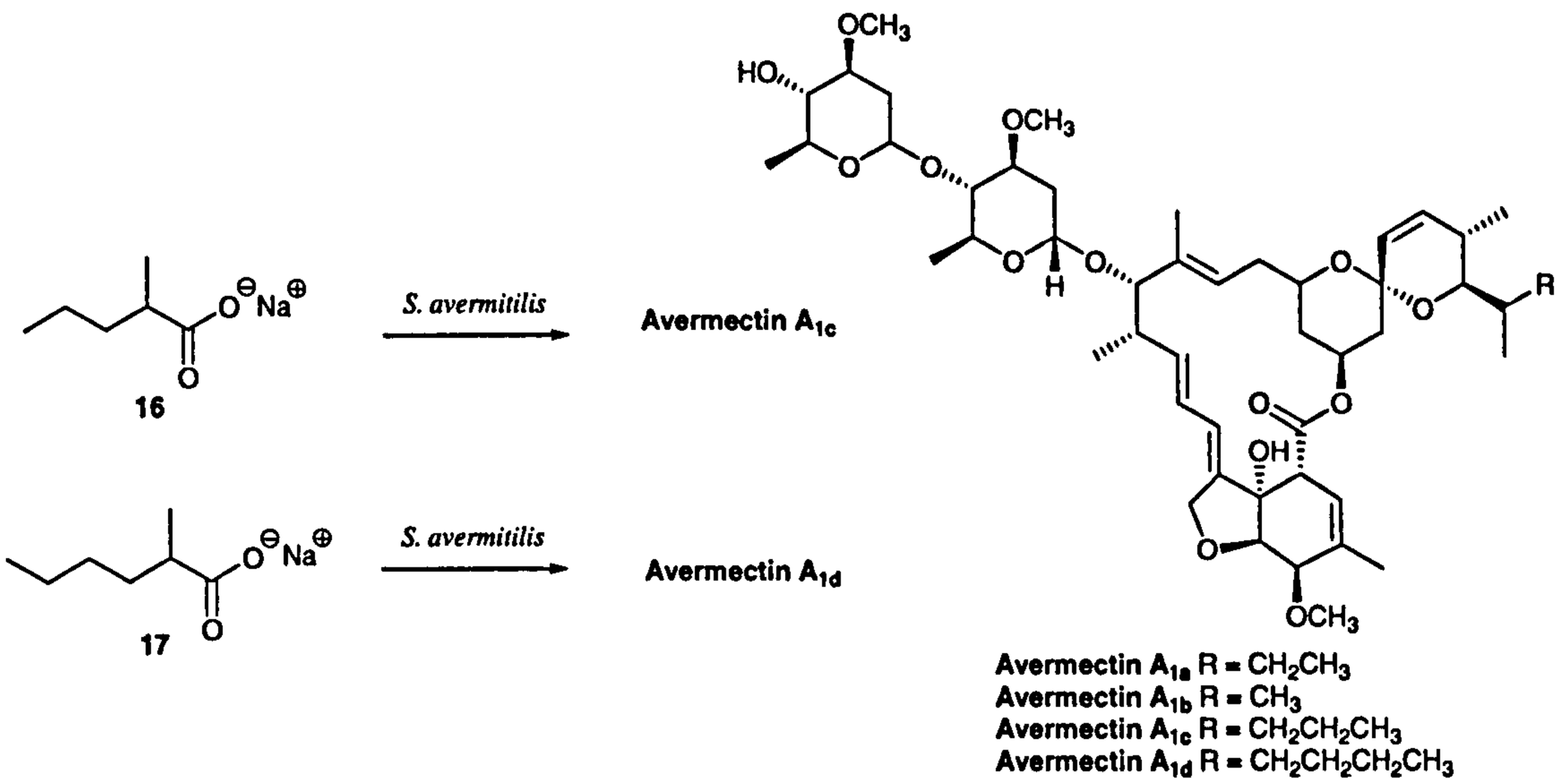
Scheme 16: Inhibition of NADPH in *S. erythreus* by GEB resulting in a switch to 6-deoxyerythronolide B production.[31]

1.3 Directing Polyketide Biosynthesis

In the literature there are many examples of research groups using their knowledge of a biosynthetic pathway in order to alter it to their advantage.[1] Some groups have even combined PKS genes from more than one source.[32,33] In this way, novel compounds can be produced *in vivo*/*in vitro* without the need for a lengthy total synthesis. There are five main approaches; precursor directed biosynthesis and mutasynthesis, domain mutagenesis, linking of modules, combining multimodular protein subunits and modifying post-PKS processing.

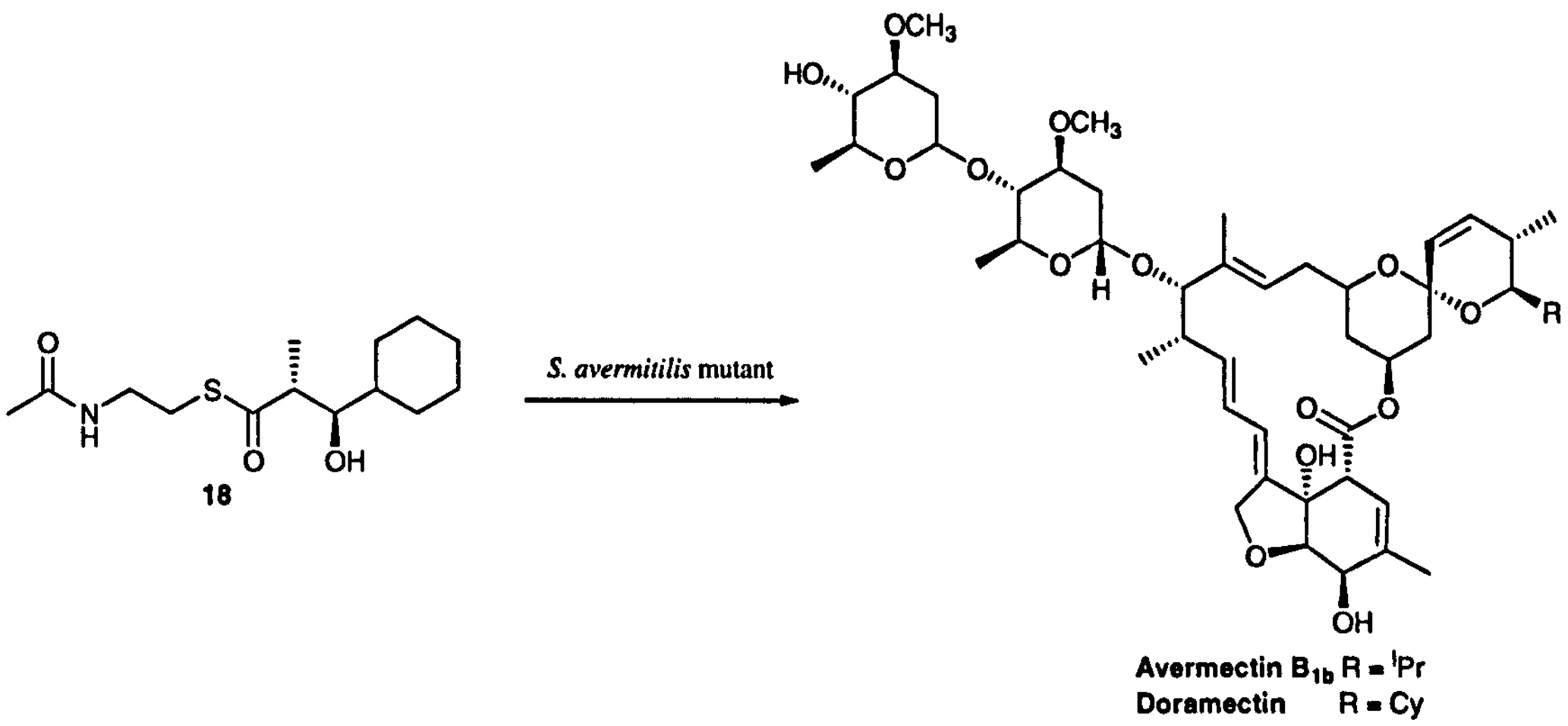
In general, enzymes are known to have narrow substrate specificity (though there are exceptions). Fortunately however, enzymes involved in the production of secondary metabolites are often less substrate specific than those responsible for primary metabolism. Precursor-directed biosynthesis involves feeding analogues of natural biosynthetic precursors to the producing organism in the hope that they will be assimilated into the final product giving an analogue of the natural product. For example, Chen *et al.* used this method to produce novel analogues of the avermectin family – known for their potent antiparasitic activity.[34] The group fed 2-methylpentanoate **16** and 2-methylhexanoate **17** starter units to *Streptomyces avermitilis* and found that they incorporated in place of the natural isobutyryl (avermectin A_{1a}) and isopropyl (avermectin A_{1b}) precursors in the metabolites formed

(Scheme 17). Unsurprisingly, the new derivatives, designated avermectin A_{1c} and avermectin A_{1d} respectively, displayed potent anthelmintic and insecticidal activity.



Scheme 17: Precursor-directed biosynthesis to produce novel avermectin analogues.[34]

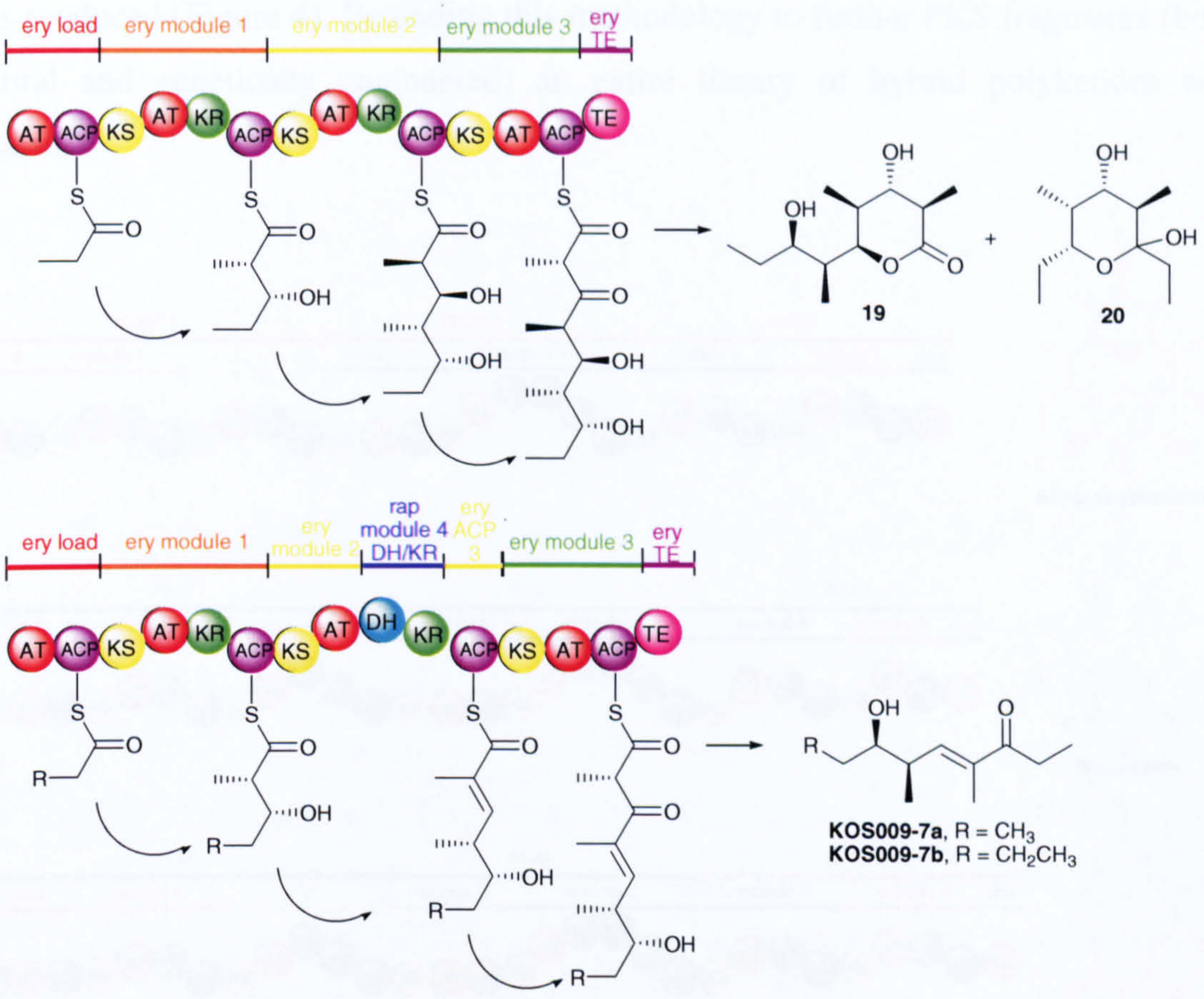
Mutasynthesis is a similar approach in which the PKS is genetically altered so as to disable the first chain extension cycle (typically by knocking out the ketosynthase domain in the first module). The mutant PKS is then supplied with a synthetic non-natural diketide or triketide intermediate in the hope that it will be processed in place of the natural starter unit. Staunton and co-workers used this approach, also working with the avermectins, to prepare a cyclohexyl derivative.[35] A mutant strain of *S. avermitilis* was prepared which lacked a branched chain ketoacid decarboxylase and was thus unable to produce the L-valine and L-isoleucine derived starter units required for polyketide production. Then a cyclohexyl derivative of a proposed biosynthetic intermediate **18** was fed to the organism and was assimilated to produce a novel avermectin, doramectin. Again, this avermectin derivative displayed high levels of antiparasitic activity and has been developed by Pfizer in their animal health product Dectomax®.



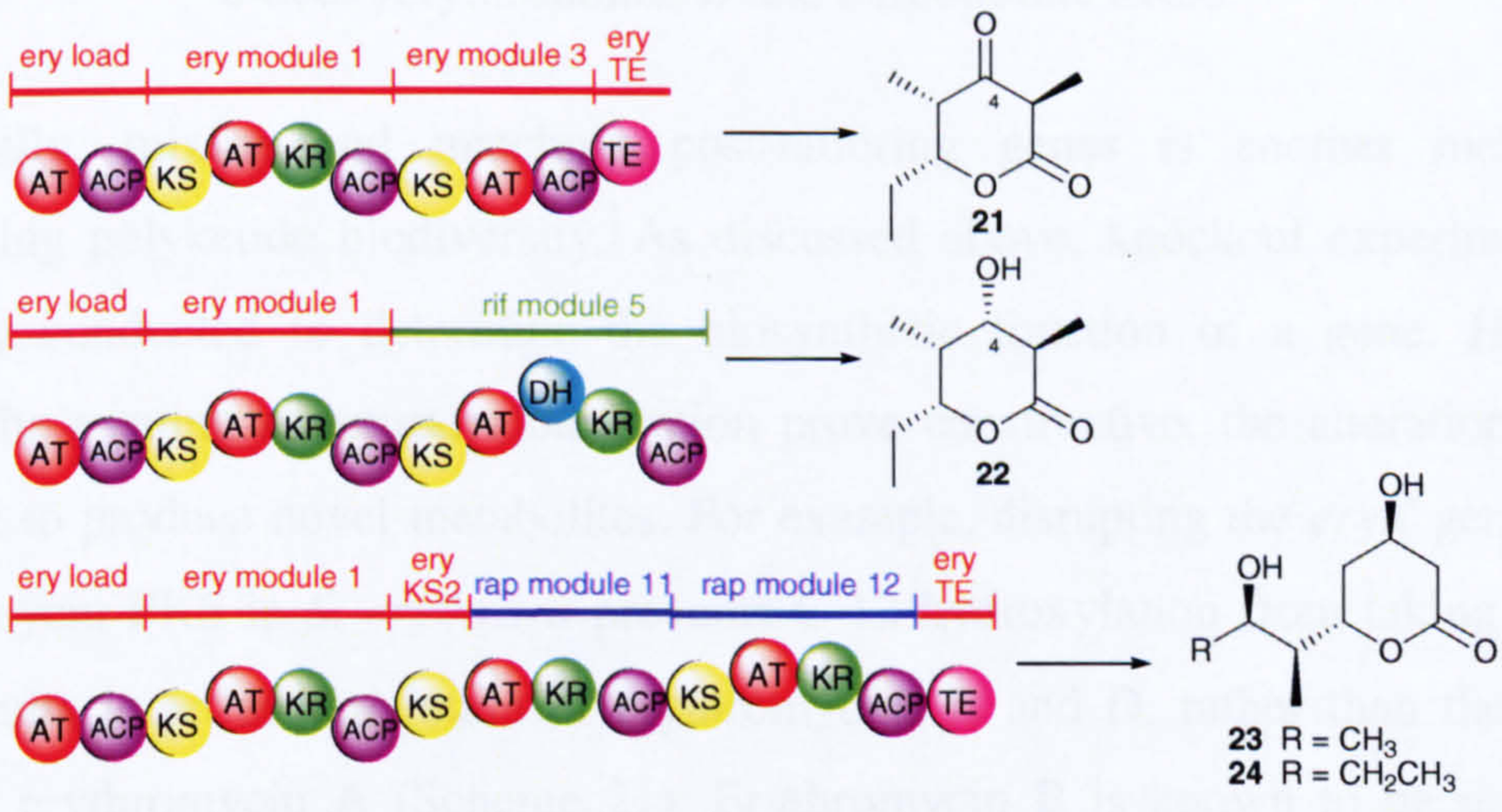
Scheme 18: Mutasynthesis to produce the novel avermectin analogue doramectin.[35]

Domain mutagenesis (i.e. swopping, inactivating or deleting individual domains) is a very versatile technique. New starter and extender units can be introduced by exchanging loading domains, the level and stereochemical outcome of ketoreduction can be altered by swopping KR domains and various reduction processes can be introduced or eliminated from the biosynthetic pathway by addition or deletion of the domains responsible. For example, McDaniel *et al.* replaced the KR of module 2 of the erythromycin PKS with the DH and KR domains of module 4 of the rapamycin PKS.[36] Instead of obtaining the tetraketide lactone **19** and hemiketal **20** previously observed for expression of the first three DEBS domains,[28] as expected, dehydrated derivatives designated KOS009-7a and KOS009-7b respectively were observed (Scheme 19).

As well as swopping individual domains, it is also possible to combine entire modules either from different locations within the same PKS or from more than one source. Khosla and co-workers successfully used this strategy to fuse module 1 of the erythromycin (DEBS) PKS to both module 3 of the same PKS and module 5 of the rifamycin PKS resulting in two lactones **21** and **22** which differed only at the C-4 position (Scheme 20).[37] Staunton and co-workers developed this methodology further, to assemble a trimodular system comprising of erythromycin and rapamycin modules, which was capable of producing simple statin analogues **23** and **24**.[38]



Scheme 19: Domain mutagenesis to include an additional reduction process.[36,28]



Scheme 20: Linking of modules from the erythromycin, rifamycin and rapamycin PKSs.[37,38]

It is also possible to combine entire multimodular proteins either in their native form or genetically modified in some way. Tang *et al.* produced a variety of novel macrocycles using this approach.[39] By combining protein fragments from the PKSs of deoxyerythronolide B (DEBS) and narbonolide (PikPKS) a novel hybrid macrolide

was produced (Figure 4). Extending this methodology to further PKS fragments (both natural and genetically engineered) an entire library of hybrid polyketides was created.

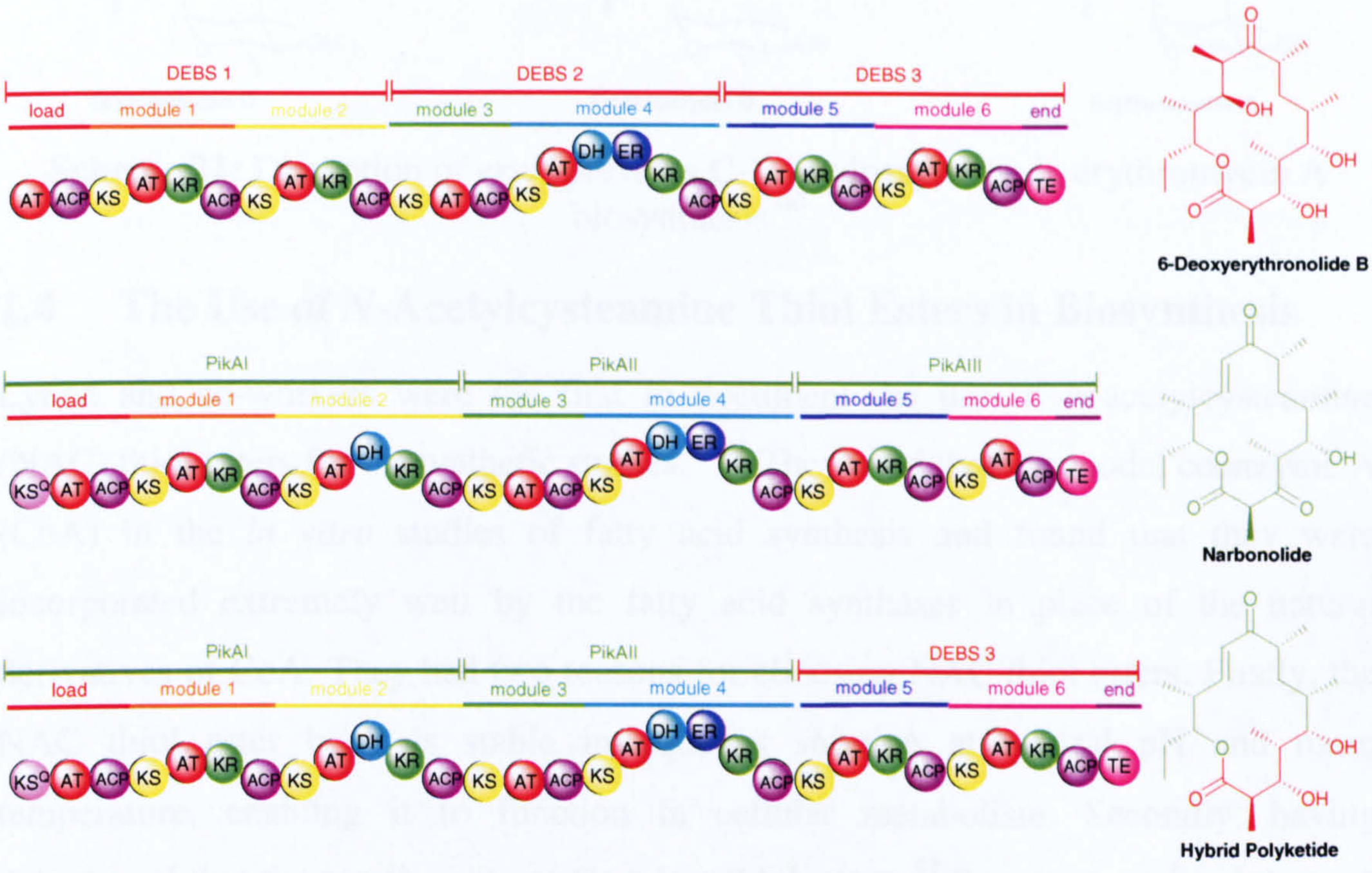
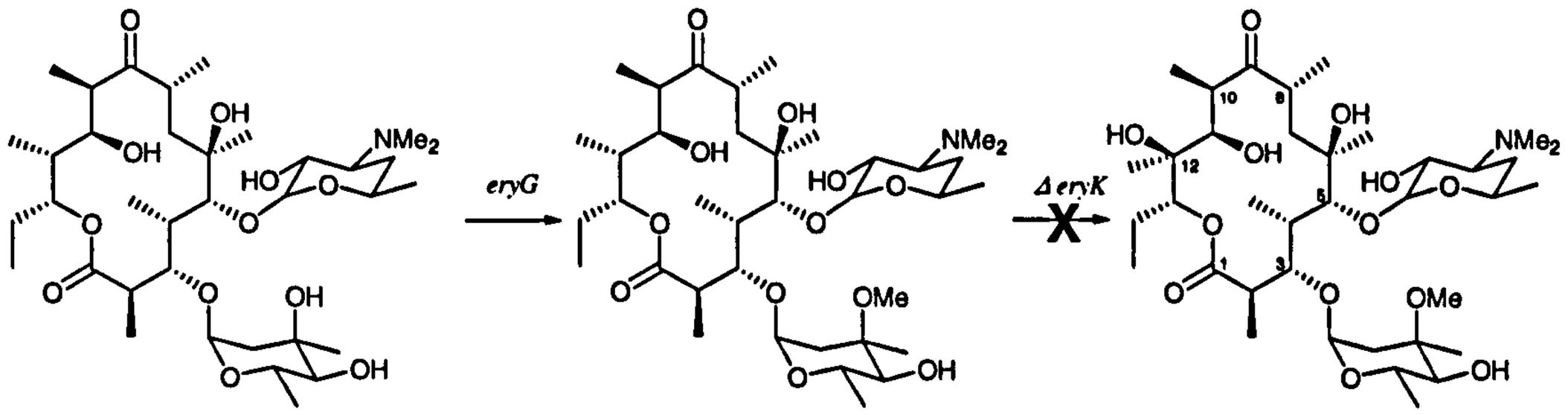


Figure 4: Hybride polyketide produced by combining protein fragments from the 6-deoxyerythronolide B and narbonolide PKSs.[39]

Finally, mixing and matching post-tailoring genes is another method of introducing polyketide biodiversity. As discussed above, knockout experiments are regularly conducted to determine the biosynthetic function of a gene. However, should the ensuing structural modification prove constructive, the alteration can be expoited to produce novel metabolites. For example, disrupting the *eryK* gene of the erythromycin PKS in *S. erythraea* prevents C-12 hydroxylation from taking place.[40] This results in an accumulation of erythromycins B and D, rather than the natural product, erythromycin A (Scheme 21). Erythromycin B is known to be more acid stable than erythromycin A (a major drawback of this commercial antibiotic, as it degrades rapidly in the stomach),[41] whilst also retaining antibacterial activity and thus may offer an alternative remedy.

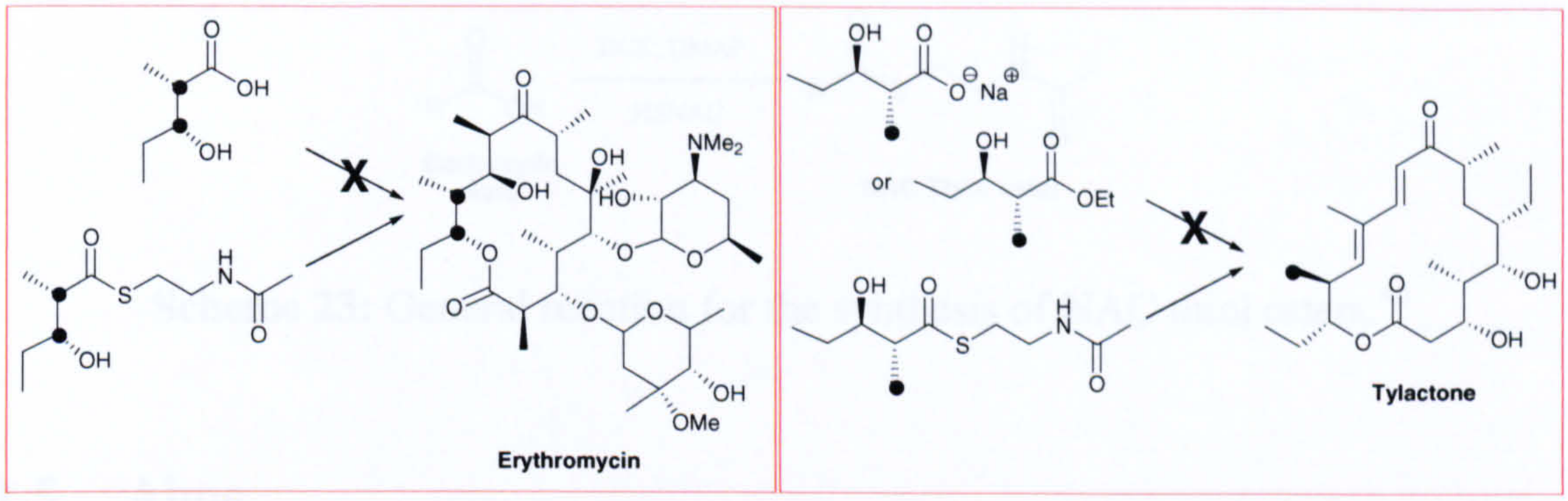


Scheme 21: Disruption of *eryK* prevents C-12 hydroxylation in erythromycin A biosynthesis.[40]

1.4 The Use of *N*-Acetylcysteamine Thiol Esters in Biosynthesis

Lynen and co-workers were the first to document the use of *N*-acetylcysteamine (NAC) thiol esters for biosynthetic studies.[42,43] They used them to model coenzyme A (CoA) in the *in vitro* studies of fatty acid synthesis and found that they were incorporated extremely well by the fatty acid synthases in place of the natural derivatives of CoA. They had two reasons for choosing NAC thiol esters. Firstly, the NAC thiol ester bond is stable in aqueous solution at neutral pH and room temperature, enabling it to function in cellular metabolism. Secondly, having determined that the reactive site of CoA is a thiol group,[42] they soon realised through experimentation, that acyl groups bound to sulfur behave differently to the corresponding esters and free acids. This is due to the difference in electronic behaviour of oxygen and sulfur. The lone pairs on the oxygen atom delocalise into the acyl group whereas the sulfur atom does not readily permit resonance stabilisation around the thiol ester linkage and so the carbonyl carbon retains its electrophilic characteristics to a greater degree, making it more prone to nucleophilic attack.

NAC thiol esters have also been shown to be recognised and processed both *in vitro* and *in vivo* by the analogous polyketide synthases. Cane *et al.*[44] and Hutchinson and co-workers[12] found they could be incorporated intact into macrolides such as erythromycin and tylactone in place of the free acids and ethyl esters, which degraded prior to incorporation (Scheme 22). They have been successfully used to probe the catalytic function of polyketide synthases from plant and bacterial origin[45] and to analyse enzyme kinetics[46] and substrate specificity.[23]



Scheme 22: Incorporation of thiol esters into the polyketide macrolides erythromycin and tylactone.[12,44]

Furthermore, NAC thiol esters have been used in place of complex thiol esters such as acyl carrier proteins (ACPs).[23,47] Comparing the structure of *N*-acetylcysteamine to that of CoA and ACP it is easy to see the structural similarities (Figure 5).

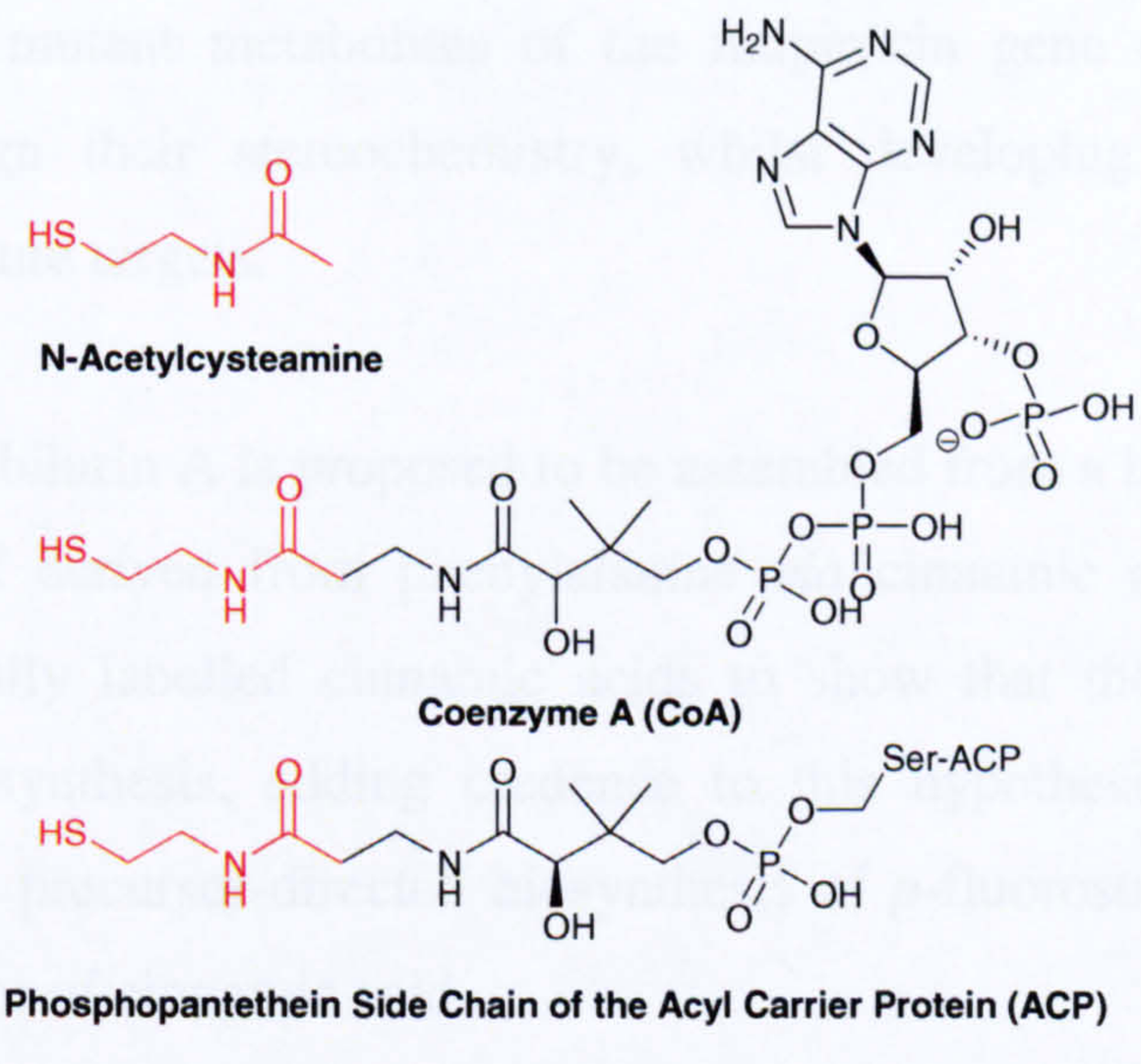
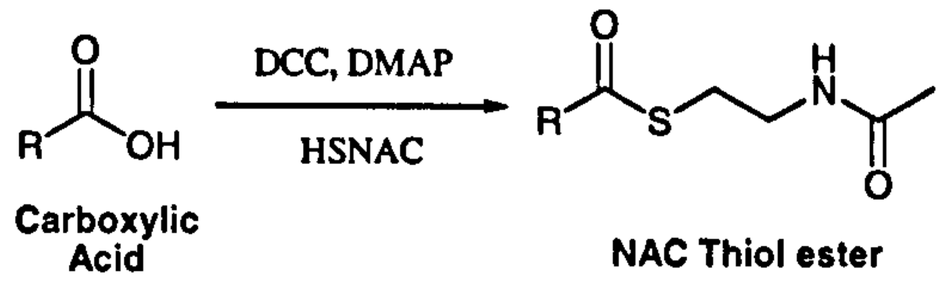


Figure 5: Comparison of structures of *N*-acetylcysteamine, CoA and ACP.

NAC thiol esters are easily prepared by condensation of the putative carboxylic acid precursor with *N*-acetylcysteamine in the presence of DCC and DMAP (Scheme 23).[48] This ease of preparation adds to their popularity as precursors for feeding studies.



Scheme 23: General reaction for the synthesis of NAC thiol esters.[48]

1.5 Aims

The overall aim of this project was to further investigate polyketide biosynthesis, focusing on three different metabolites – the pseudomonic acids (mupirocin), strobilurin A and alternapyrone.

The Pseudomonic Acids: Using isotopically labelled thiol esters we hoped to perform feeding studies with *Pseudomonas fluorescens* NCIMB 10586 to establish the origins of the 9-hydroxynonanoic acid side chain of pseudomonic acid A. We also wanted to prepare mutant metabolites of the mupirocin gene cluster in order to unequivocally assign their stereochemistry, whilst developing flexible synthetic methodology for future targets.

Strobilurin A: Strobilurin A is proposed to be assembled from a benzoyl-CoA starter unit which is itself derived from phenylalanine *via* cinnamic acid. We aimed to synthesise isotopically labelled cinnamic acids to show that the C-2–C-3 bond is cleaved during biosynthesis, adding credence to this hypothesis. In addition, we sought to explore a precursor-directed biosynthesis of *p*-fluorostrobilurin A using a fluorinated derivative of cinnamic acid.

Alternapyrone: Our objective was to prepare putative alternapyrone precursors for feeding studies with cell-free extracts of PKSN (a PKS isolated from *Alternaria solani*). In particular, our goal was to investigate the *C*-methylation process which occurs in all but the third catalytic cycle.

Chapter 2

The Pseudomonic Acids: Isotopic Labelling Studies

2.1 The Pseudomonic Acids

The pseudomonic acids are polyketide derived secondary metabolites, produced by *Pseudomonas fluorescens*, (a diverse species of bacteria that is commonly found in a wide range of terrestrial and aquatic habitats), which show varying degrees of antibiotic activity.[49,50]

The basic structure of all the pseudomonic acids is a C_{17} monic acid entity with a C_9 fatty acid side chain (Figure 6).[51] The four most abundant pseudomonic acids are pseudomonic acid A,[51] pseudomonic acid B, which has an additional hydroxyl group at C-8,[52] pseudomonic acid C, which has a double bond in place of the epoxide group at C-10–C-11[53] and pseudomonic acid D, which has an alkene group at C-4′–C-5′ in the side chain.[54] The extract of wild-type *P. fluorescens* NCIMB 10586 comprises of a mixture of pseudomonic acids (90% pseudomonic acid A, 8% pseudomonic acid B and <2% of pseudomonic acids C and D) and is given the generic name "mupirocin",[54] although mupirocin is also frequently used to refer solely to the major metabolite, pseudomonic acid A.

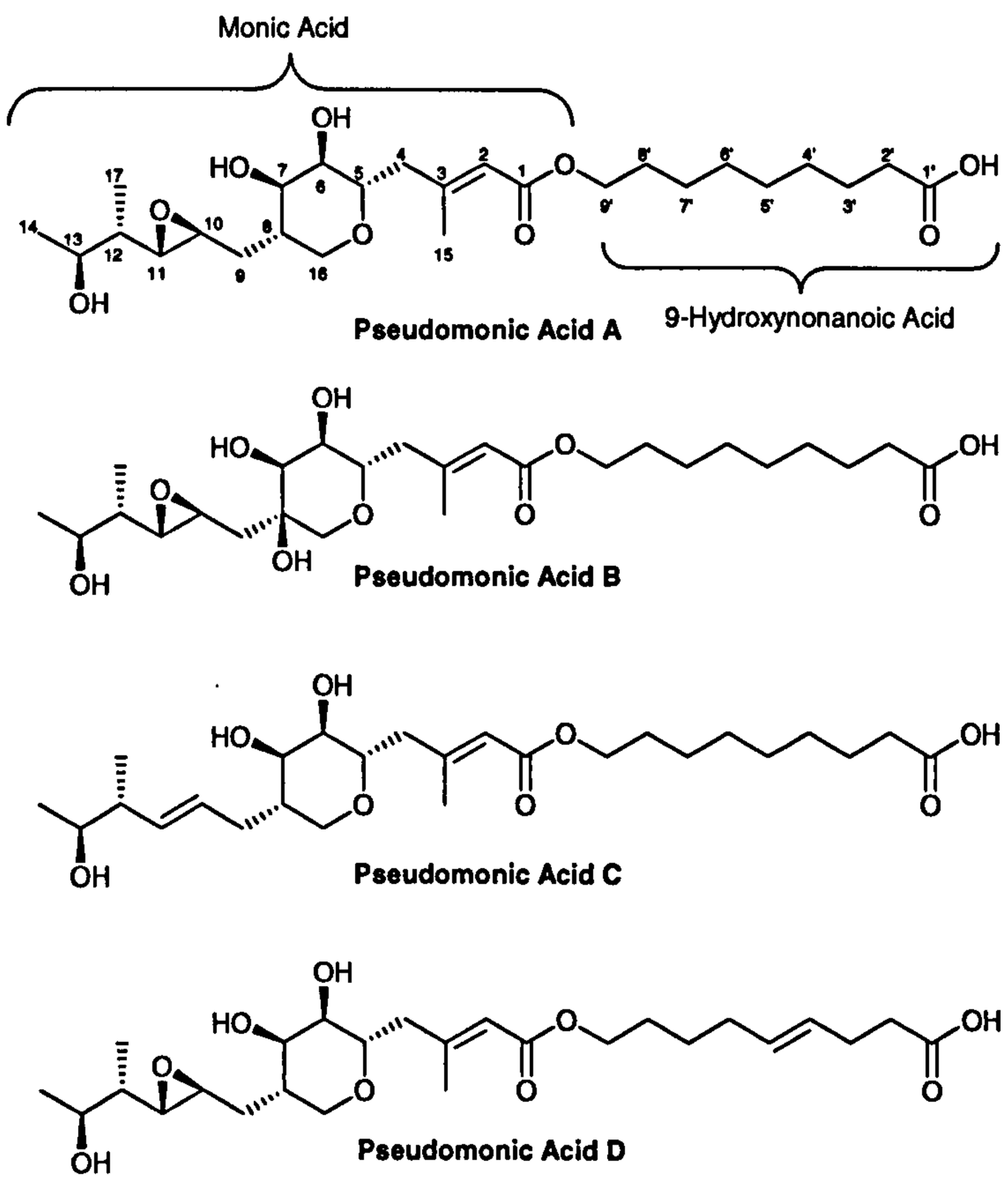
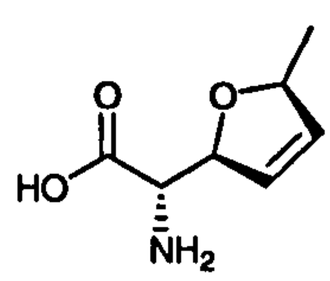


Figure 6: The pseudomonic acids.

Mupirocin is the active pharmaceutical ingredient of Bactroban® and Bactroban Nasal® (originally developed by Beecham and now marketed by GlaxoSmithKline). These topical and intranasal ointments are used clinically to treat a variety of bacterial skin infections and as a means of controlling *Staphylococcus aureus* (in particular methicillin-resistant *Staphylococcus aureus*), succeeding where other antibiotics have failed.[55,56]

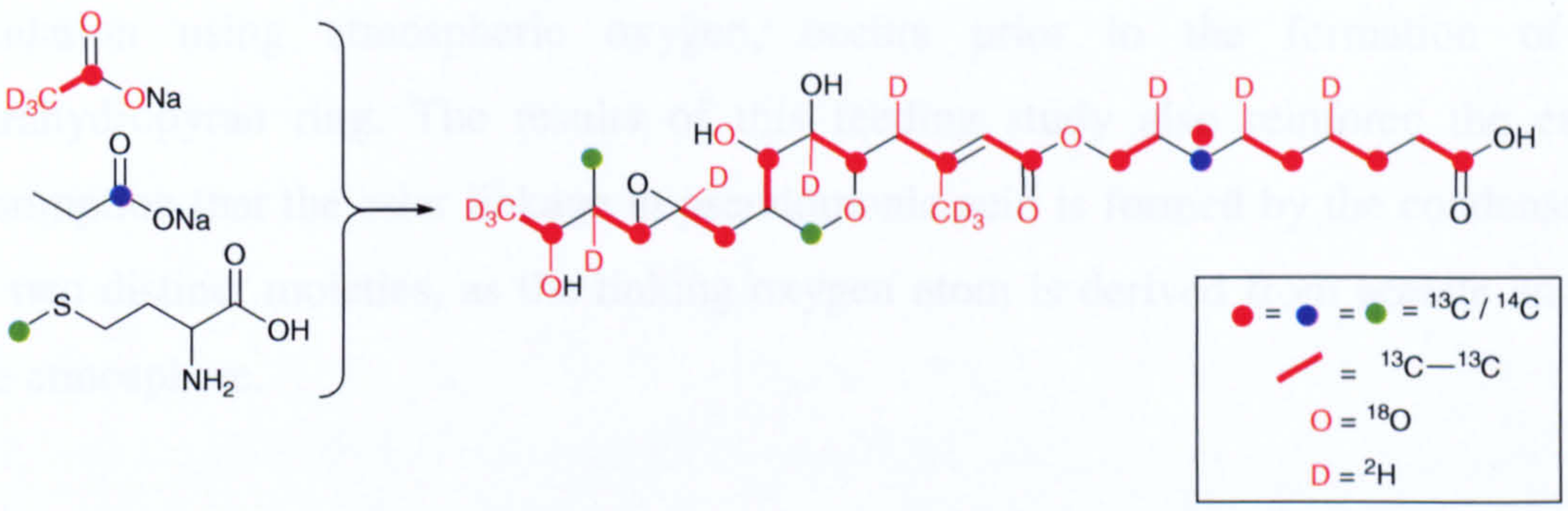
Mupirocin is a potent inhibitor of Gram positive bacteria. It is believed to act by blocking the activity of bacterial isoleucyl-tRNA synthase.[57] This enzyme is necessary for protein synthesis. Without it, the bacterial cell is unable to manufacture vital cell proteins or form protective cell walls. Most antibiotics act on either bacterial DNA or the cellular walls of bacteria. Mupirocin is only the second naturally occurring inhibitor of bacterial isoleucyl-tRNA synthase to be discovered, the first being furanomycin.[58,59] Its unusual mode of action means it shows no cross resistance with antibiotics such as penicillin, streptomycin, methicillin, erythromycin and many other common antibiotics. The pseudomonic acids and their derivatives are therefore important pharmaceutical targets and have been the subject of many total syntheses.[60,61,62]



Furanomycin

2.2 Isotopic Labelling Studies on the Pseudomonic Acids

Several labelling studies have been successfully carried out on the pseudomonic acids.[63,64] [1-^{14}C]-, [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]-acetates all showed good incorporation into pseudomonic acid A, accounting for the origins of the carbon backbone (Scheme 24). The incorporation pattern has also revealed some aspects of the biosynthetic pathway. For instance, the labelling of both C-1 and C-9′ by [1-^{13}C]-acetate discounts the possibility of the ester linkage forming *via* a Baeyer-Villiger-type oxidation of a preformed acetate-derived aliphatic chain. Instead it suggests the condensation of two distinct biosynthetic units. The incorporation of [2-^{13}C]-acetate at C-15, shows that unlike C-17, it is derived from a cleaved acetate unit and not *S*-adenosyl methionine (SAM), suggesting that this moiety was also enzymatically constructed from two distinct segments.[63]



Scheme 24: Labelling pattern of pseudomonic acid from acetate, propionate and methionine precursors.[63,64]

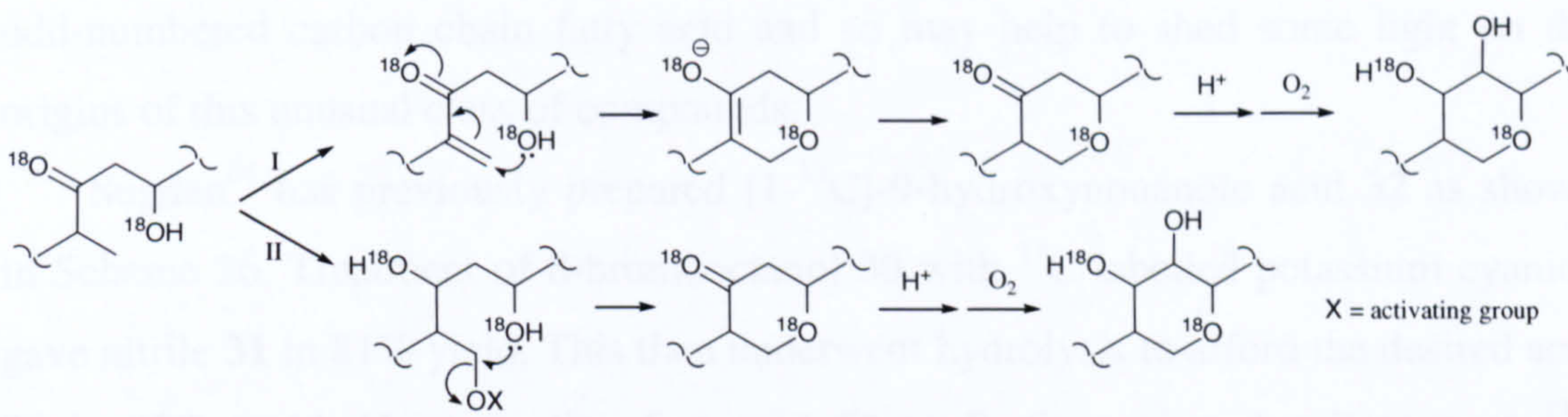
[1-^{13}C]-Propionate incorporated solely at the C-7′ position (though this position was also labelled from C-1 of acetate). This, coupled with the fact that the C-8′–C-9′ acetate unit is not incorporated in the usual head to tail fashion observed for polyketides, again suggests the union of two separate biosynthetic units (most likely the starter unit which primes the 9-hydroxynonanoic acid side chain). However, although [2-^{14}C]- and [3-^{13}C]-propionate were also incorporated, they did so at positions which indicated that degradation to [1-^{14}C]- and [2-^{13}C]-acetate respectively had occurred prior to assimilation. This seems to suggest that propionate is not the actual starter unit for the 9-hydroxynonanoic acid side chain, merely a precursor to it.

D,L-[Me-^{14}C]-Methionine and L-[Me-^{13}C]-methionine were both incorporated into pseudomonic acid A verifying the source of the one-carbon branches at C-16 and C-17. The failure of [^{14}C]-formate (another "C_1 pool" donor) to incorporate reinforced this assignment.

[1-^{13}C, $^{2}H_3$]-Acetate was fed to *P. fluorescens* by Martin and Simpson[64] in order to determine the biosynthetic origins of the hydrogen atoms. By measuring the β-shifts in the ^{13}C NMR spectrum they were able to ascertain the number of deuterium atoms bonded to the adjacent carbon atom (Scheme 24). No deuterium was found on C-8, (though this is in agreement with the proposed biosynthesis of the polyketide backbone in which C-8 is fully unsaturated – see Scheme 38, Chapter 3), but no explanation was proposed for the lack of deuterium at C-2 or C-2′.

Incorporation of [1-^{13}C, $^{18}O_2$]-acetate revealed the origins of the oxygen atoms of pseudomonic acid A (Scheme 24). From the results of these labelling studies it has been postulated that ring closure is effected by attack of the 5-hydroxyl group either onto an enone, I, or onto C-16 with a good leaving group, II (Scheme 25). It is also entirely possible that 6-hydroxylation, which presumably arises as a result of an

oxidation using atmospheric oxygen, occurs prior to the formation of the tetrahydropyran ring. The results of this feeding study also reinforce the earlier assumption that the ester linkage in pseudomonic acid is formed by the condensation of two distinct moieties, as the linking oxygen atom is derived from acetate and not the atmosphere.



Scheme 25: Proposed mechanisms for the formation of the tetrahydropyran ring.

Many possible precursors to pseudomonic acid have been ruled out by incorporation studies. Substrates such as [3-^{14}C]-3-hydroxy-3-methylglutarate **25**,[64,65] [3,6-$^{13}C_2$]-3-hydroxy-3-methylglutarate **26**,[64] [1,5-$^{14}C_2$]-3-hydroxy-3-methylglutarate **27**,[65] (*RS*)-[2-^{14}C]-mevalonic acid **28**[63] and diethyl [1,2-$^{13}C_2$]-malonate **29**[66] (Figure 7) either failed to incorporate or showed multiple label incorporation indicating that they had degraded completely to acetate prior to incorporation.

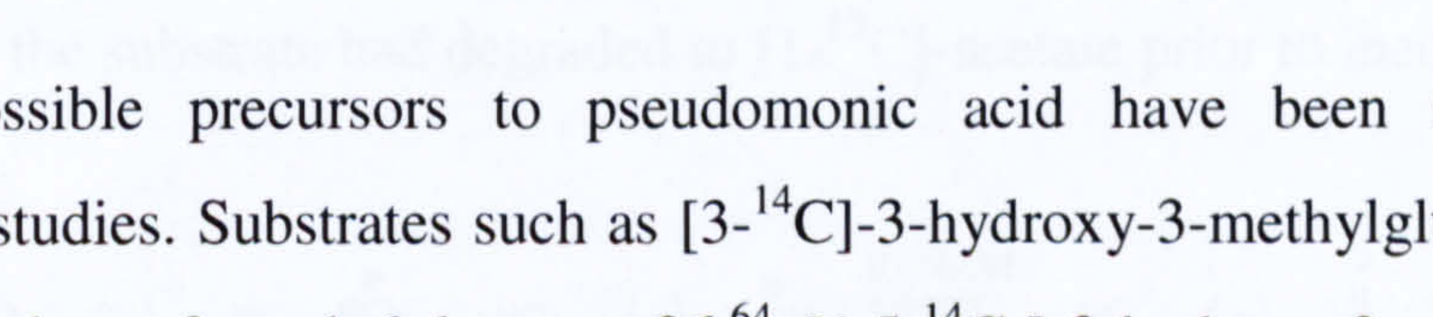


Figure 7: Isotopically labelled compounds which failed to incorporate into pseudomonic acid. [63,64,65,66]

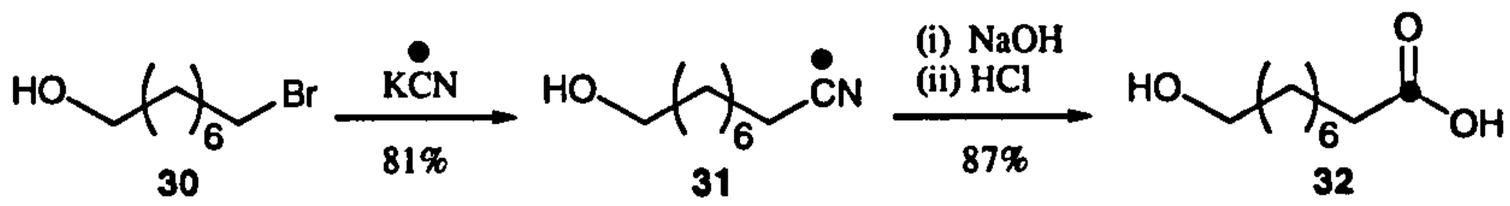
2.3 Investigation of 9-Hydroxynonanoic Acid as an Intermediate in Mupirocin Biosynthesis

As discussed above, many different feeding studies have been performed on the pseudomonic acids over the years and much has been ascertained about their biosynthetic origins and their metabolic mode of assembly. However, there are still many puzzling and unexplained results to be investigated and theories to be confirmed experimentally.

One such hypothesis is that the pseudomonic acids are derived from two distinct units - a monic acid moiety and a 9-hydroxynonanoic acid entity. However, incorporation of labelled 9-hydroxynonanoic acid and monic acid into pseudomonic acid has never been formally demonstrated.

As well as its relevance to the biosynthesis of pseudomonic acid, 9-hydroxynonanoic acid is also important in its own right, as it is a rare example of an odd-numbered carbon chain fatty acid and so may help to shed some light on the origins of this unusual class of compounds.

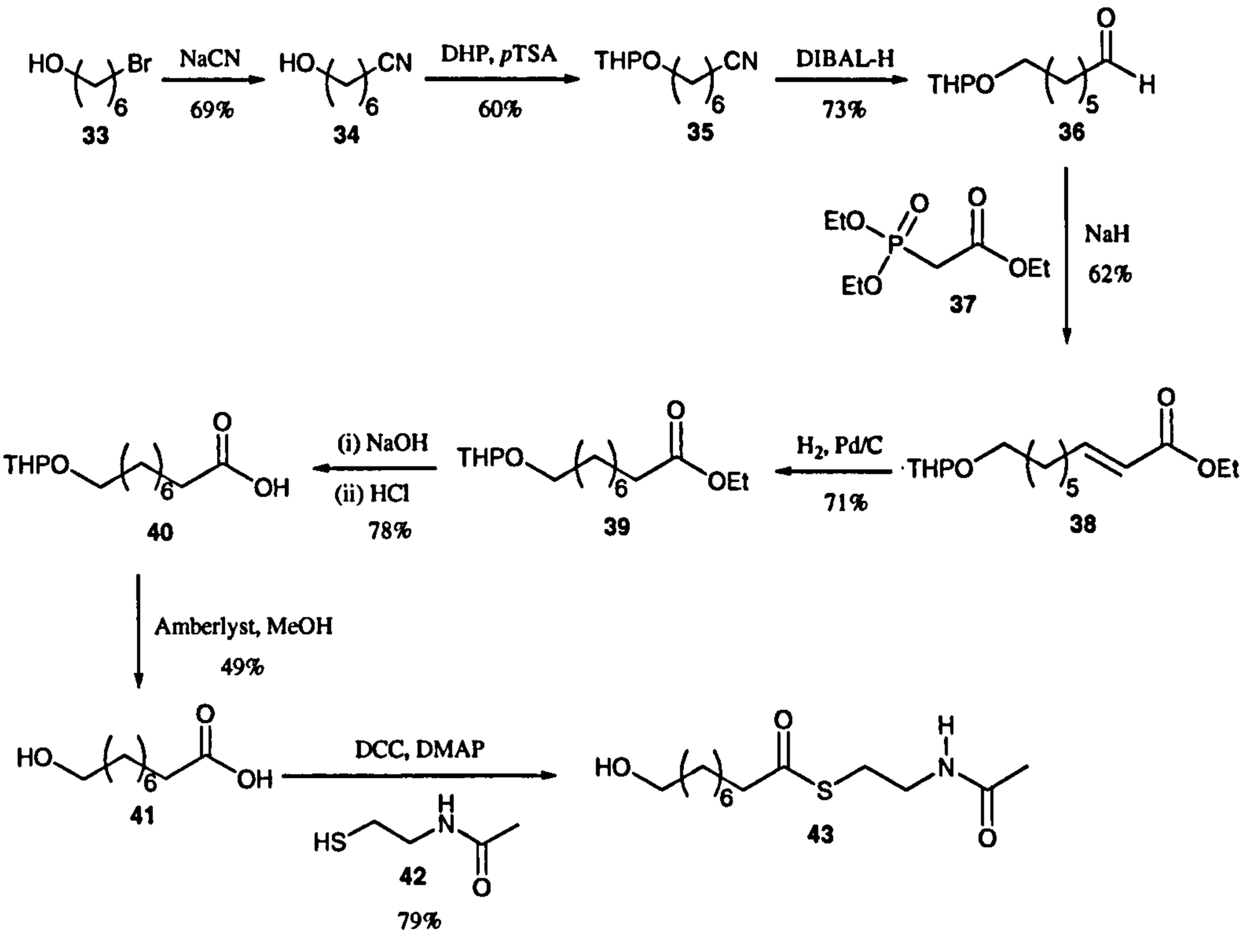
Sugden[66] has previously prepared [1-^{13}C]-9-hydroxynonanoic acid **32** as shown in Scheme 26. Treatment of 8-bromooctanol **30** with ^{13}C labelled potassium cyanide gave nitrile **31** in 81% yield. This then underwent hydrolysis to afford the desired acid **32** in 87% yield. Upon feeding free acid **32** to *P. fluorescens* he discovered ^{13}C incorporation at C-1′, C-3′, C-5′, C-7′, C-9′, C-1, C-3, C-5, C-7, C-9, C-11 and C-13 indicating that the substrate had degraded to [1-^{13}C]-acetate prior to incorporation.



Scheme 26: Sugden's route to [1-^{13}C]-9-hydroxynonanoic acid **32.**[66]

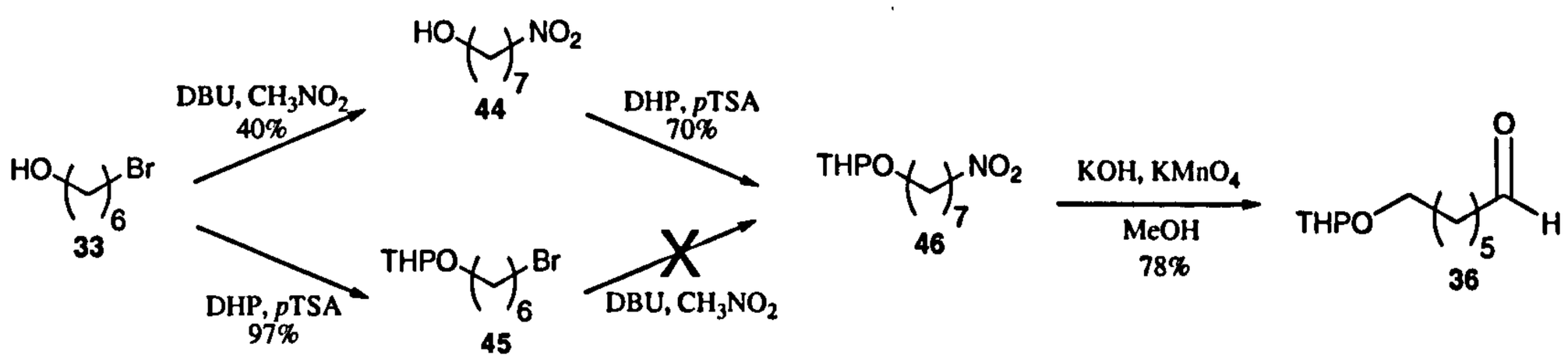
As described in section 1.4, there are several examples in the literature of thiol esters acting as substrates for feeding studies in situations where the free acid has failed to incorporate. It was therefore decided to prepare the corresponding NAC thiol ester with two vicinal ^{13}C atoms to increase the sensitivity of label detection in the product post feeding.

Unlabelled 9-hydroxynonanoic NAC acid thiol ester **43** has previously been prepared on a very small scale by Donlevy[67] (Scheme 27). Treatment of 6-bromo-1-hexanol **33** with sodium cyanide gave **34,** followed by protection of the hydroxyl group and reduction of the nitrile functionality in **35** furnished aldehyde **36** in 73% yield. This then underwent a Horner-Wadsworth-Emmons chain elongation with triethyl phosphonoacetate **37** to yield the unsaturated ester **38.** Hydrogenation of the olefin followed by hydrolysis of the resulting ester, furnished acid **40** which was then deprotected and coupled to *N*-acetylcysteamine **42** to furnish thiol ester **43** in 79% yield.



Scheme 27: Donlevy's final synthetic route to the thiol ester **43**.[67]

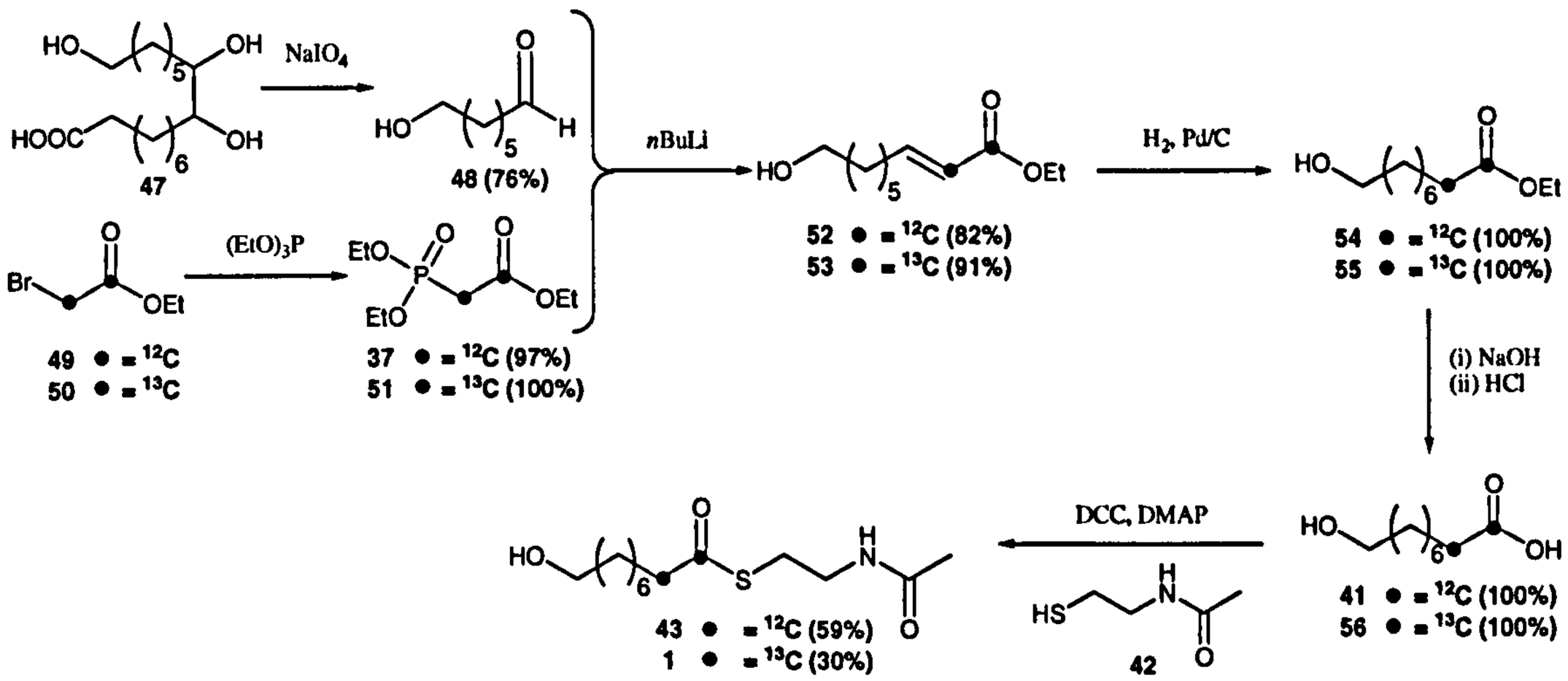
Our initial route was based upon that of Donlevy but a slightly different synthesis of aldehyde **36**, which avoided the use of cyanide, was employed. Using the same starting material, 6-bromo-1-hexanol **33** and following the general procedure of Bäckvall *et al.*,[68] chain extension by addition of nitromethane was investigated. The reaction did not proceed at room temperature but when heated to reflux furnished nitroheptanol **44**. The latter was then converted to aldehyde **36** by protection of the hydroxyl group and Nef reaction of the resulting nitroalkane **46** (Scheme 28). Unfortunately, this route was low yielding due to the formation of several by-products during the addition of nitromethane (possibly due to the elevated temperatures required), although enough of the protected aldehyde **36** was prepared to continue the synthesis for another two steps, yielding the unsaturated ester **39**. Performing the addition and protection steps in the opposite order only served to decrease the yields further. Nef reaction of **45** produced an array of inseparable products and so it became clear that a new route was required.



Scheme 28: Synthesis of aldehyde **36** *via* Nef reaction of nitroalkane **46**.

It was interesting to speculate if a hydroxyl protecting group was really required at all throughout the synthesis. Concerned that such a flexible chain might be prone to cyclisation/polymerisation reactions, Donlevy's original plan had been to remove the protecting group at the end of the synthesis. However, having completed his synthesis of the protected 9-hydroxynonanoic acid NAC thiol ester, he found the thiol group was unstable to the acidic conditions required to remove the tetrahydropyran group. He attempted the final step without a protecting group and did not encounter any problems. We therefore decided to try the entire synthesis without a protecting group.

A one step synthesis of 7-hydroxyheptanal **48** from aleuritic acid **47** was reported by Pawar *et al.* during their synthesis of the insect pheromone ferrulactone II.[69] A natural product isolated from shellac, aleuritic acid **47** underwent oxidative cleavage with sodium periodate to yield 7-hydroxyheptanal **48** in 76% yield. Reaction of aldehyde **48** with triethyl phosphonoacetate **37** furnished unsaturated ester **52** in 82% yield which was subsequently reduced quantitatively to the saturated ester **54**. Hydrolysis of ester **54** in refluxing sodium hydroxide furnished 9-hydroxynonanoic acid **41**. Finally, a DCC/DMAP mediated coupling of acid **41** with *N*-acetylcysteamine **42** furnished thiol ester **43** in 59% yield (Scheme 29). The optimised route was then used to prepare [1,2-$^{13}C_2$]-9-hydroxynonanoic acid NAC thiol ester **1**, as shown, by using commercially available ethyl [1,2-$^{13}C_2$]-bromoacetate **50** (and later [1,2-$^{13}C_2$]-triethyl phosphonoacetate **51** when this became available) to introduce the labels. A disappointingly low overall yield of 39% was achieved, due to the formation of a *N*,*S*-diacetylcysteamine contaminant in the final coupling step.



Scheme 29: Optimised route for the preparation of thiol esters **43** and **1.**

2.4 Biosynthesis of Pseudomonic Acid A by *Pseudomonas fluorescens* NCIMB 10586

As described in section 2.1, mupirocin is produced by *Pseudomonas fluorescens* NCIMB 10586. This strain was used throughout this work for feeding studies of putative precursors. Before attempting any feeding studies, however, it was necessary to establish the production pattern of mupirocin, and so a growth study was conducted. This involved growing the bacteria under the conditions described in the experimental section (chapter 7) and monitoring the levels of mupirocin production at regular intervals by HPLC.

In order to quantify the pseudomonic acid A content in the extracts, volumetric standards of mupirocin were prepared and run in duplicate on the HPLC. A peak corresponding to pseudomonic acid A was observed at 21.5 minutes (Figure 8). By running solutions of varying concentration, we were able to correlate the data into a linear graph, from which samples of unknown concentration could be determined (Figure 9). The graph was found to be linear in the range investigated (0.0078 – 0.250 mg/mL) and so provided the concentration of the samples fell between these two limits, their concentration could be determined accurately.

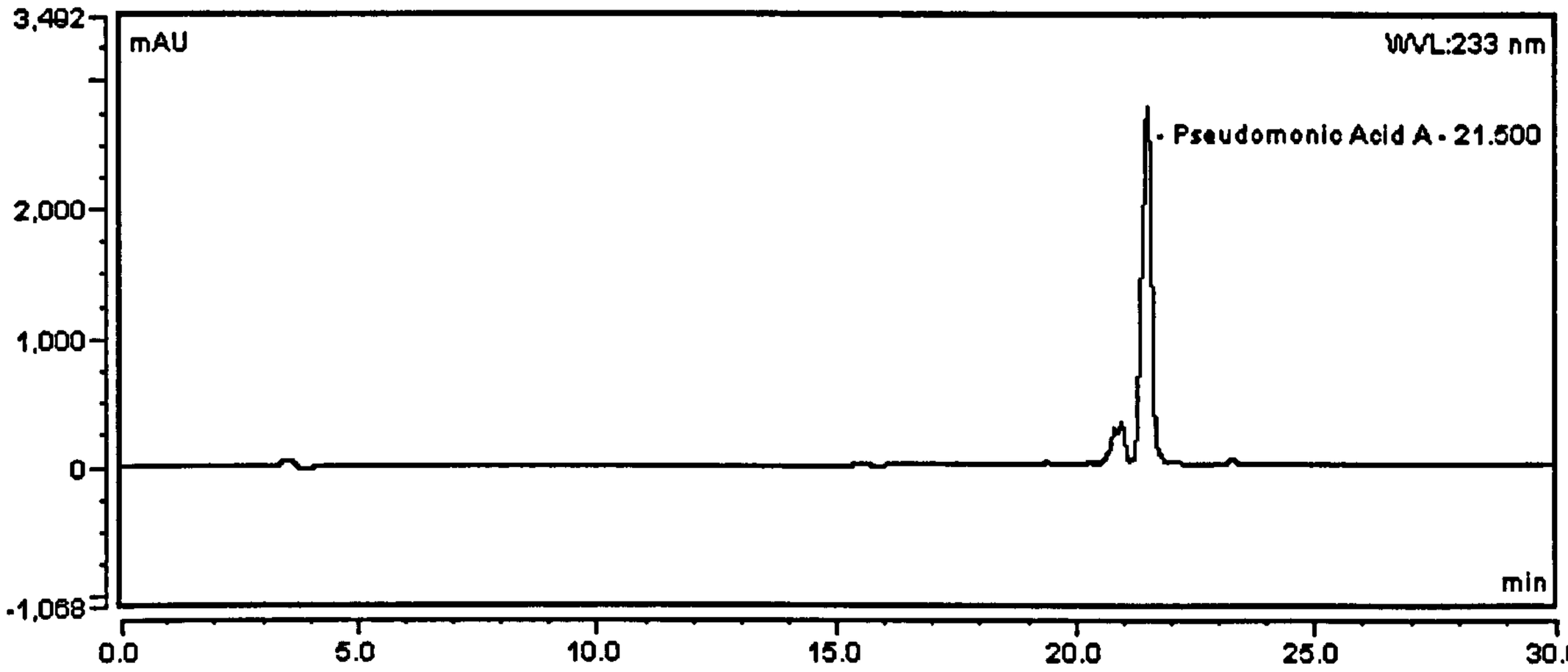


Figure 8: HPLC trace of mupirocin standard (1 mg/mL volumetric solution).

Samples of the fermentation broth were taken every two hours, starting ten hours after inoculation of the secondary stage medium. 1 mL aliquots were taken from two flasks at each interval, combined, microcentrifuged and then the supernatant was analysed immediately by HPLC. The pH of each of the samples was also measured. This enabled the growth production and pH variance curves of pseudomonic acid A to be constructed (Figure 10 and Figure 11).

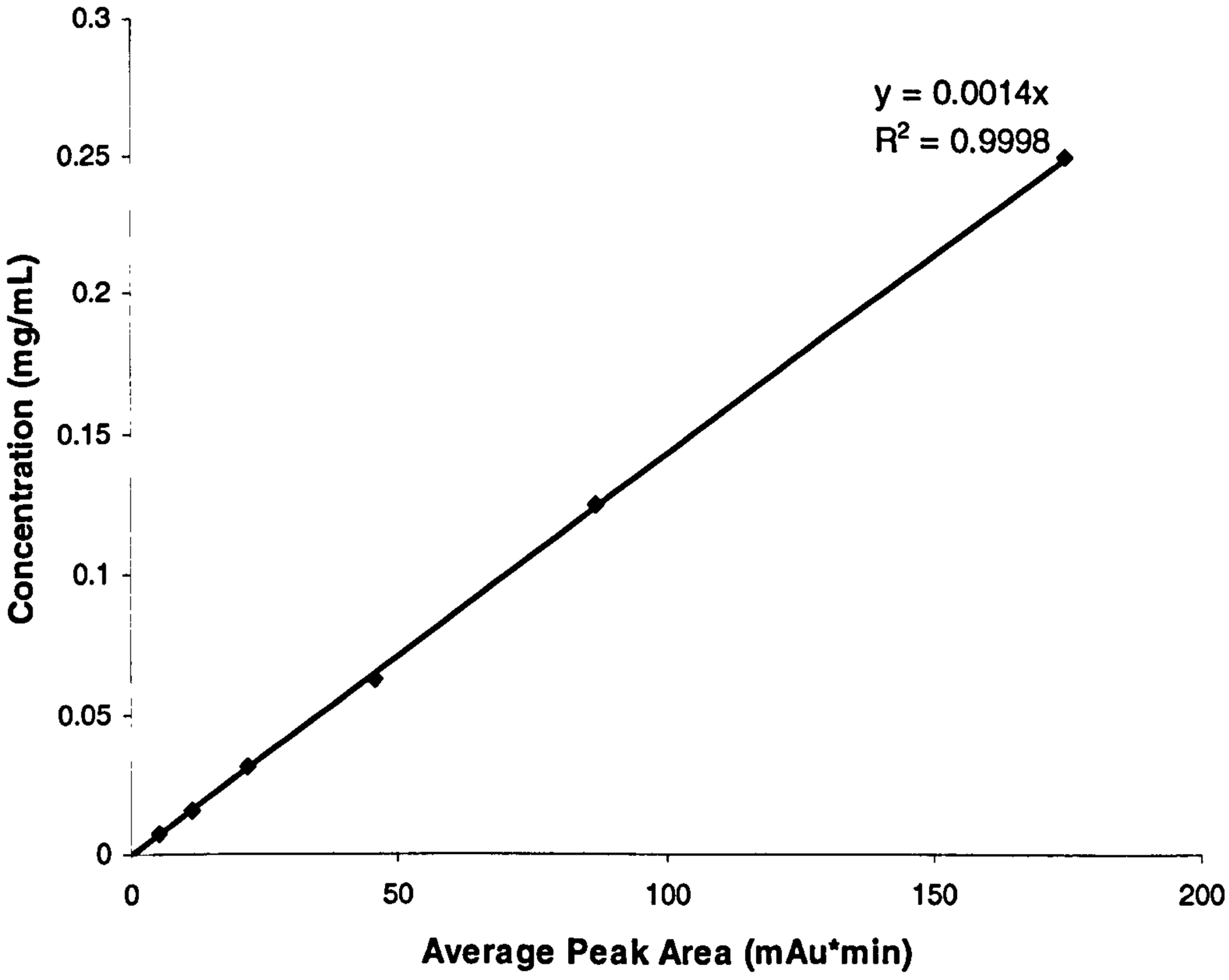


Figure 9: Mupirocin quantification graph.

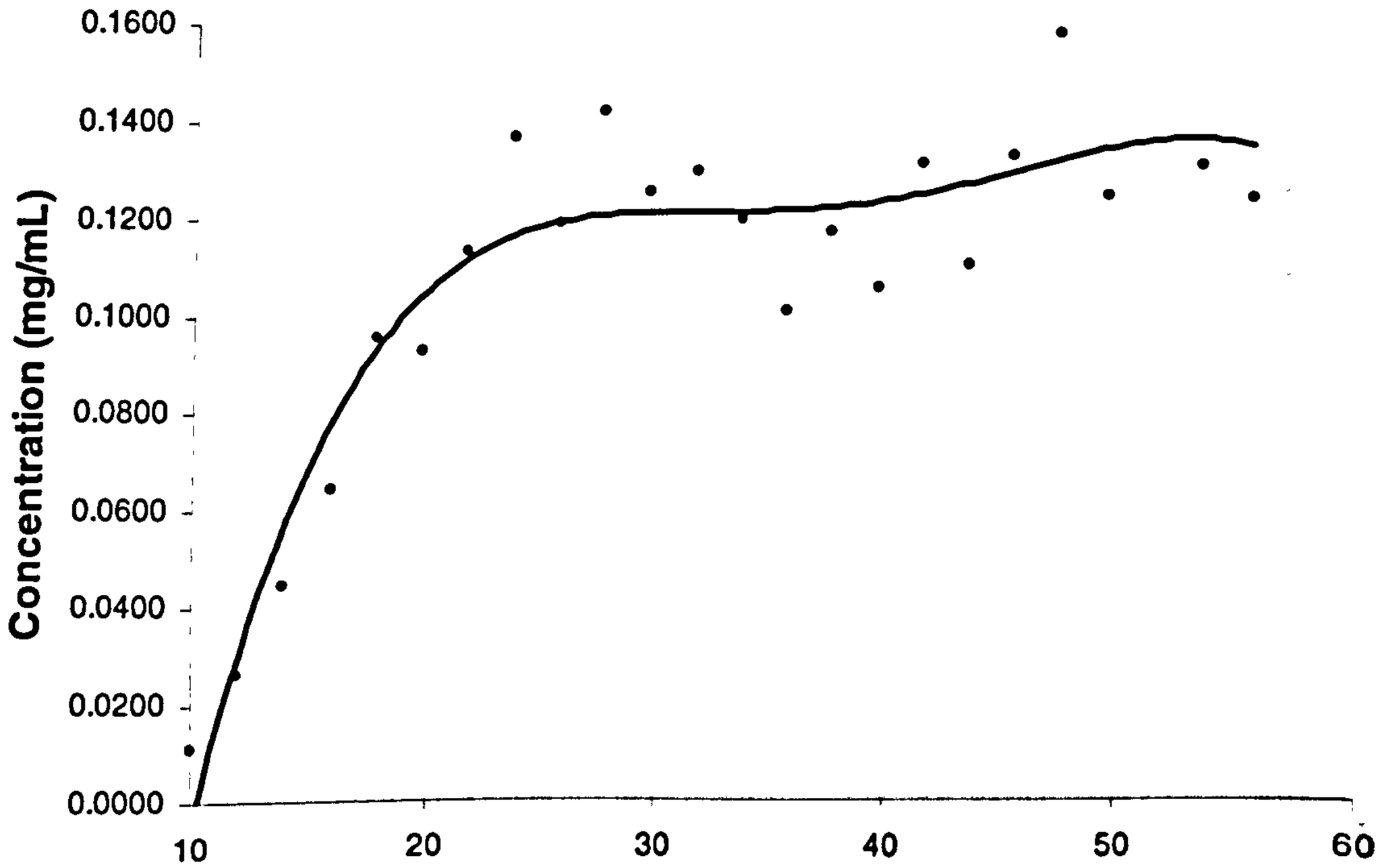


Figure 10: Growth production curve of pseudomonic acid A.

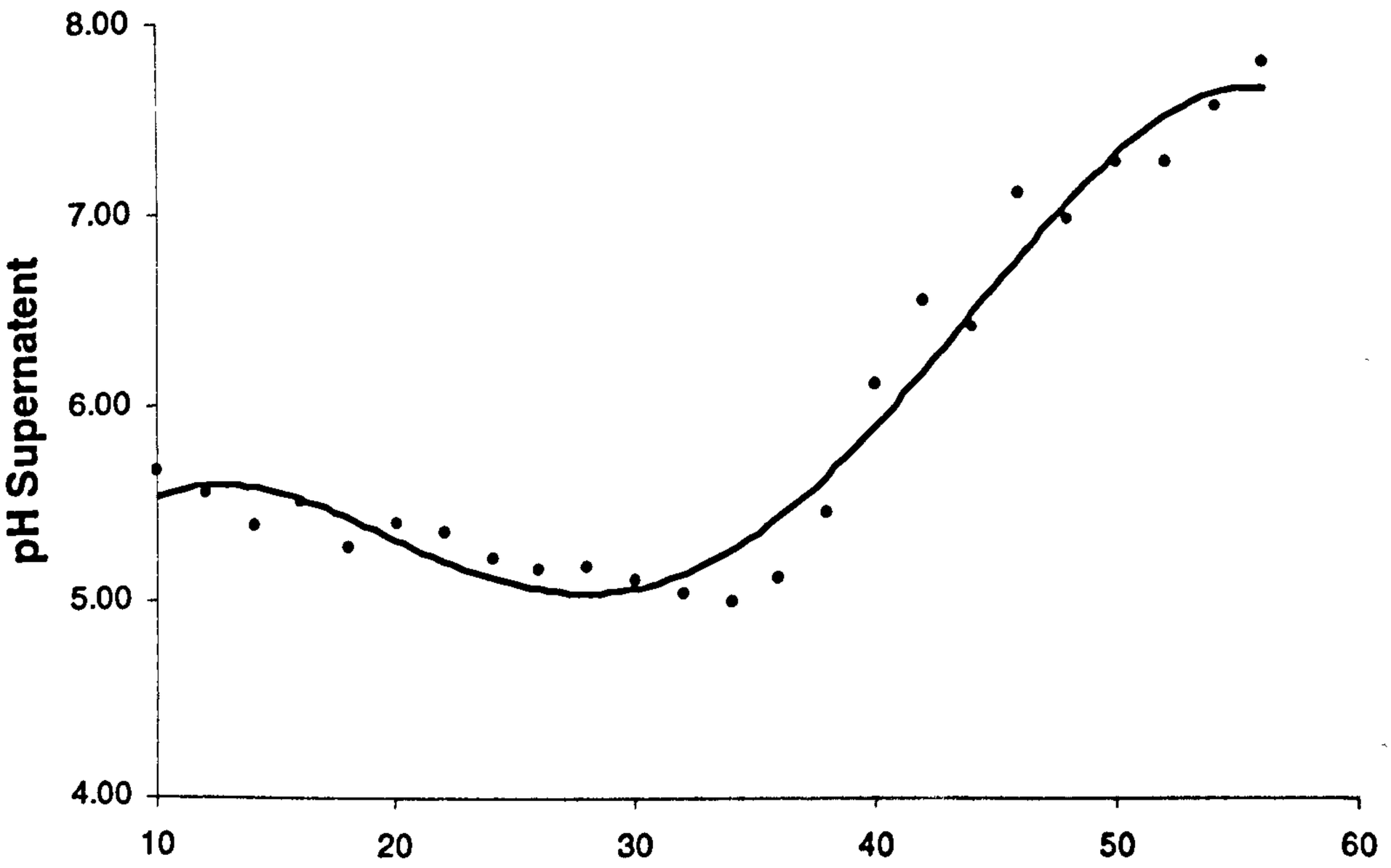


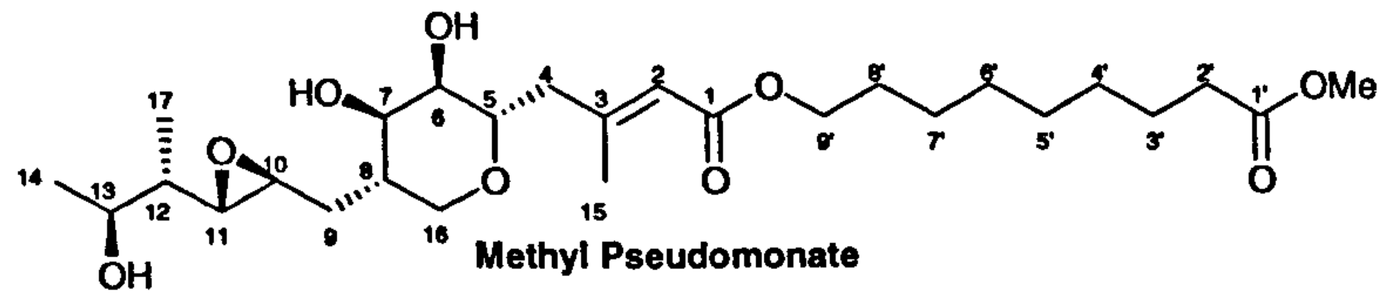
Figure 11: pH variation of fermentation broth with respect to time.

As shown in Figure 10, pseudomonic acid production commenced approximately 10 hours after inoculation of the secondary stage medium and so this is the point at which we would begin to pulse feed our precursor to the bacteria. A maximum of 0.162 mg/mL was reached at approximately 50-52 hours and so isolation of the secondary metabolite was conducted at this time point. The culture was extracted with ethyl acetate, methylated with diazomethane and purified by Sephadex® and silica column chromatography to afford 40 mg (0.04 mg/mL) of methyl pseudomonate.

The quantity of pseudomonic acid A produced by the bacteria during this study was much lower than previous reports. Martin[70] achieved unisolated yields (as determined by HPLC) of approximately 0.40 mg/mL, as did Donlevy.[67] However, it should be noted that they only succeeded in isolating approximately 0.13 and 0.06 mg/mL respectively. Laosripaiboon[71] attained HPLC yields of 0.07-0.10 mg/mL and an isolated yield of 0.01 mg/mL. Thomas and co-workers currently achieve isolated yields of approximately 0.02 mg/mL.[72] *P. fluorescens* is known to be temperamental and yields can vary dramatically from medium to medium and over time.[70,71]

Yields decrease further when a substrate is added to the culture. In order to investigate the effect of feeding on mupirocin yields, sodium acetate solutions of various concentration were pulse fed to *P. fluorescens* at five one hour intervals, starting at 10 hours. Samples were taken at three different time points (four in the case of the control) and analysed by HPLC. As the concentration of substrate was increased, the yield of mupirocin decreased. When a concentration of 250 mg/L was employed, the yield of mupirocin was approximately half the control value. Even at a concentration of 50 mg/L the yield of mupirocin had decreased by about a third.

The ^{1}H and ^{13}C NMR spectra of methyl pseudomonate (more stable than pseudomonic acid) have been assigned by Mellows and co-workers[63] and Everett and Tyler[73] (Figure 12 and Figure 13) and thus isotopic label incorporation can be detected by comparison with the reference spectra.



δ	multiplicity	H
0.94	d	17-H_3
1.22	d	14-H_3
1.31-1.38	br s	4′-H_2, 5′-H_2, 6′-H_2, 7′-H_2, 12-H
1.44-1.98	m	3′-H_2, 8′-H_2, 9-H_2
2.03	m	8-H
2.22	s	4-*H*H, 15-H_3
2.31	t	2′-H_2
2.56-2.64	m	4-H*H*
2.68-2.73	dm	11-H
2.81	m	10-H
3.46-3.59	m	6-H, 16-*H*H
3.67	s	OCH_3
3.94-3.74	m	7-H, 5-H, 13-H, 16-H*H*
4.07	t	9′-H_2
5.76	s	2-H

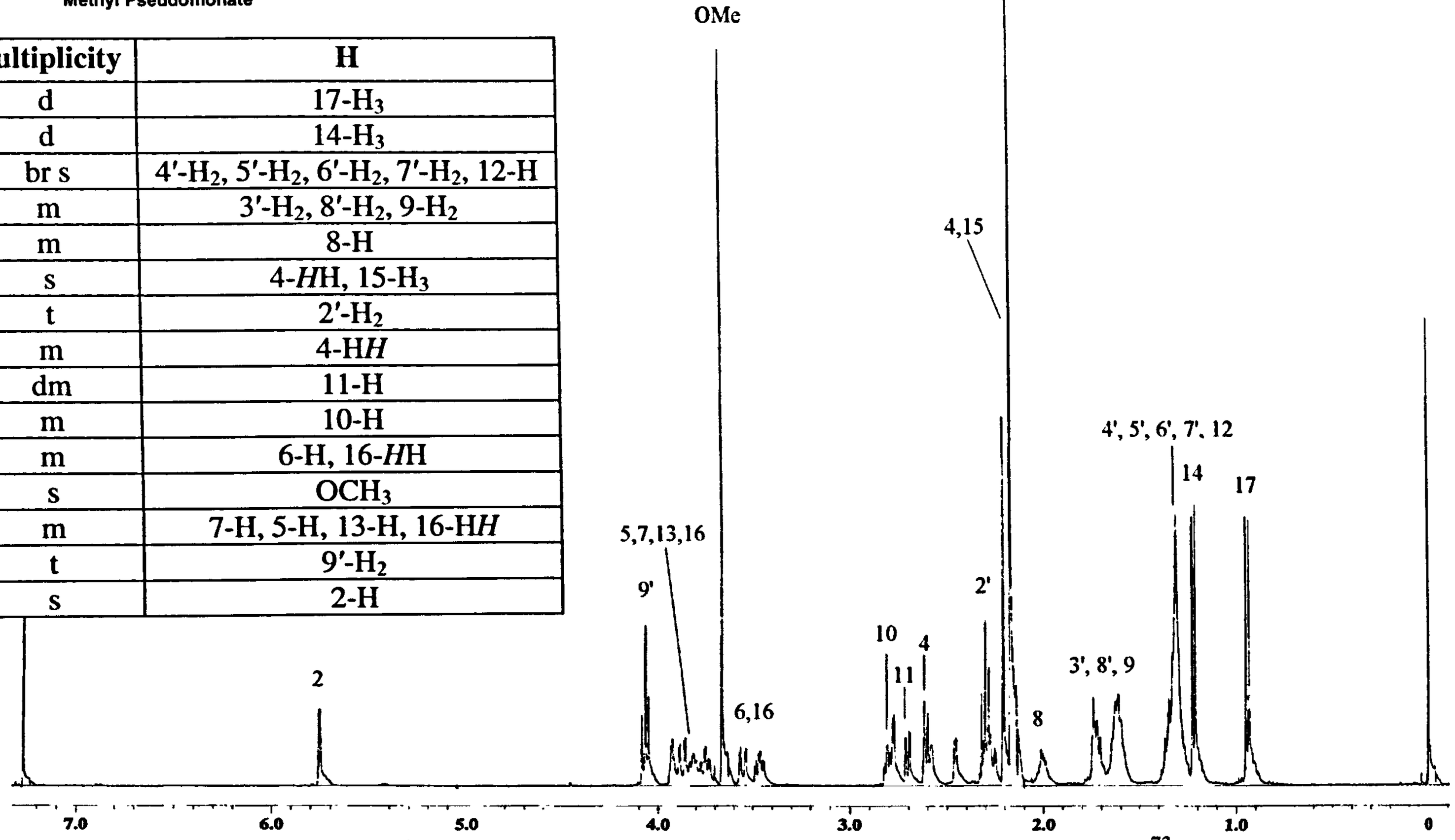
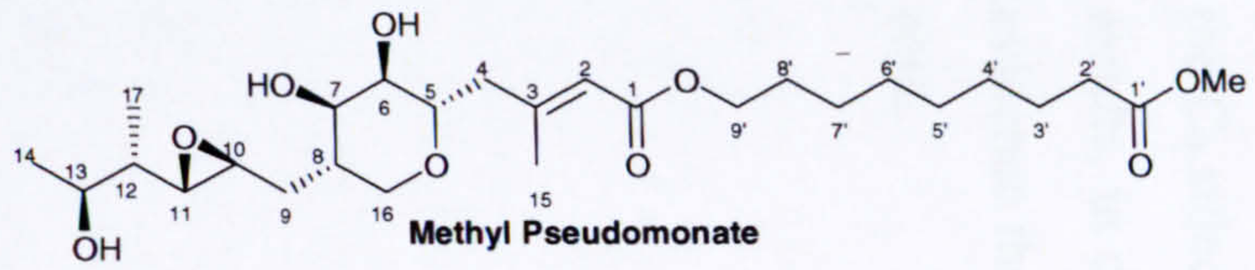


Figure 12: ^{1}H NMR spectrum and assignments of methyl pseudomonate.[73]



δ	C	δ	C
12.7	C-17	55.6	C-10
19.1	C-15	61.3	C-11
20.8	C-14	63.9	C-9′
24.9	C-3′	65.4	C-16
25.9	C-7′	69.0	C-6
28.7	C-8′	70.4	C-7
29.0	C-4′, C-5′, C-6′	71.4	C-13
31.6	C-9	74.9	C-5
34.1	C-2′	117.6	C-2
39.5	C-8	156.7	C-3
42.9	C-4, C-12	166.8	C-1
51.5	OCH_3	174.4	C-1′

Figure 13: ^{13}C NMR spectrum and assignments of methyl pseudomonate.[63]

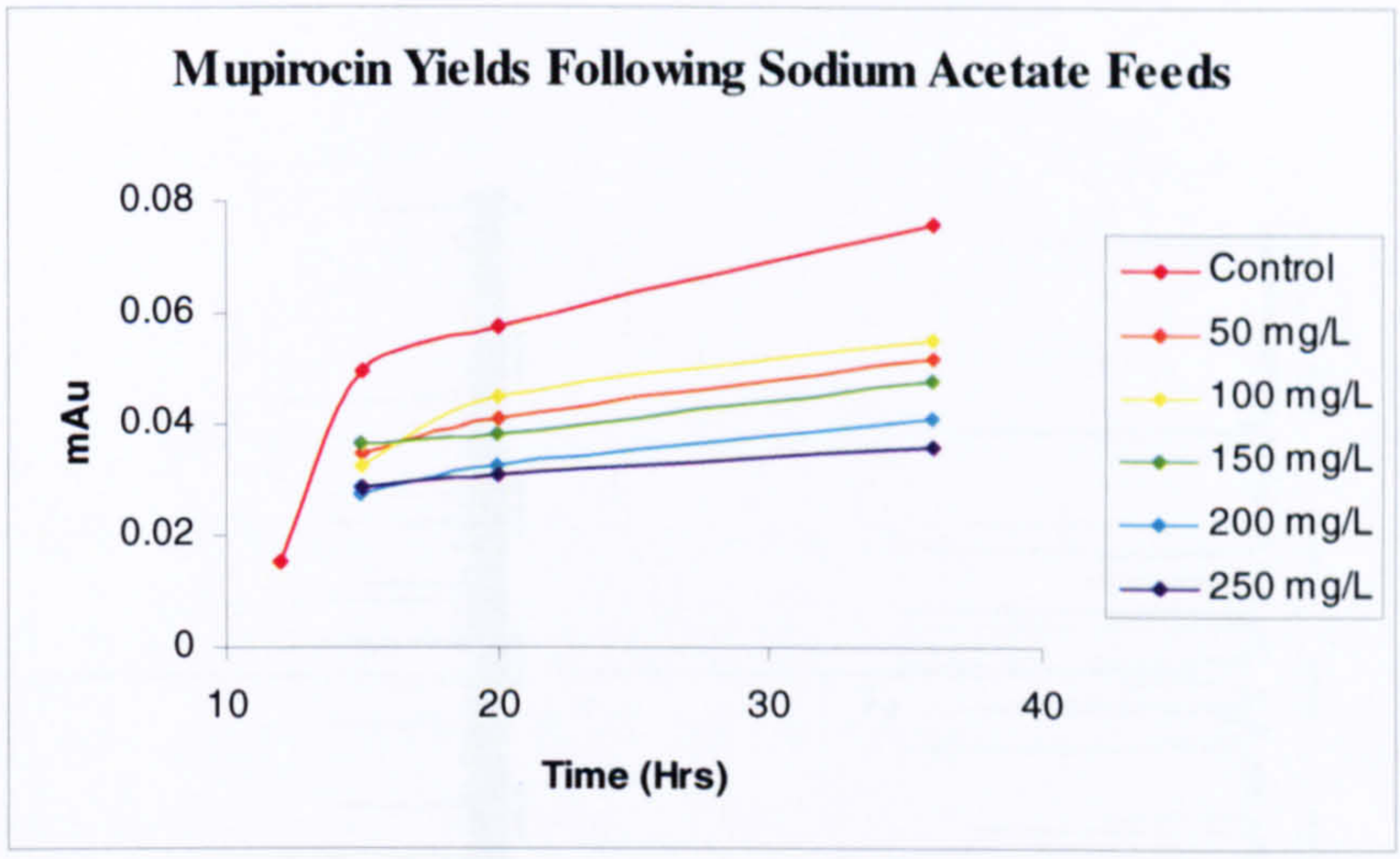


Figure 14: The effect of feed substrate concentration on the yield of mupirocin.

Thiol ester **1** was then fed to *P. fluorescens* and 18 mg of pseudomonic acid were isolated. Unfortunately, no ^{13}C incorporation was observed in the ^{13}C NMR spectrum (Figure 16). In particular, no satellite signals were observed adjacent to the C-1′ or C-2′ signals (even after 40,000 scans) to indicate ^{13}C-^{13}C coupling. Thus, we cannot conclude anything from this experiment other than that thiol ester **1** has not incorporated on this occasion.

However, metabolites recently isolated by Wu from various *mupW* mutants (J. Wu, unpublished results) seem to suggest that this result might in fact be legitimate. Acids **57** and **58**, which have C_5 and C_7 sidechains respectively, seem to suggest that the C_9 sidechain might be elongated post esterification rather than beforehand. These results, in conjunction with the failed free acid feeds of Sugden[66] provide mounting evidence that 9-hydroxynonanoic acid may in fact not be a precursor to pseudomonic acid.

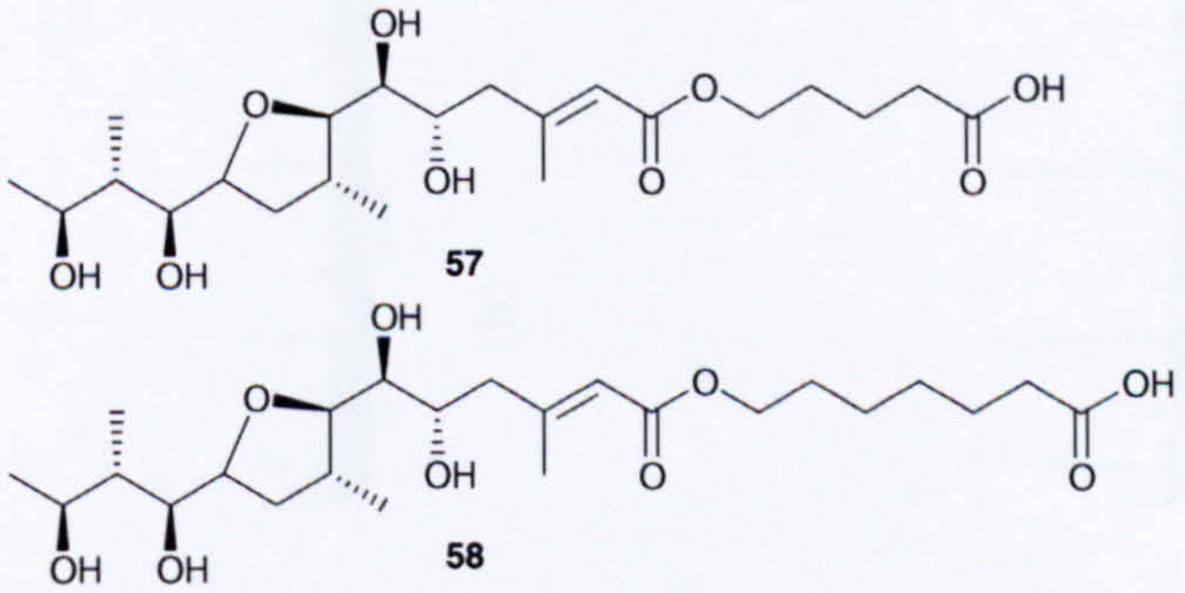


Figure 15: *MupW* mutants with truncated side-chains.

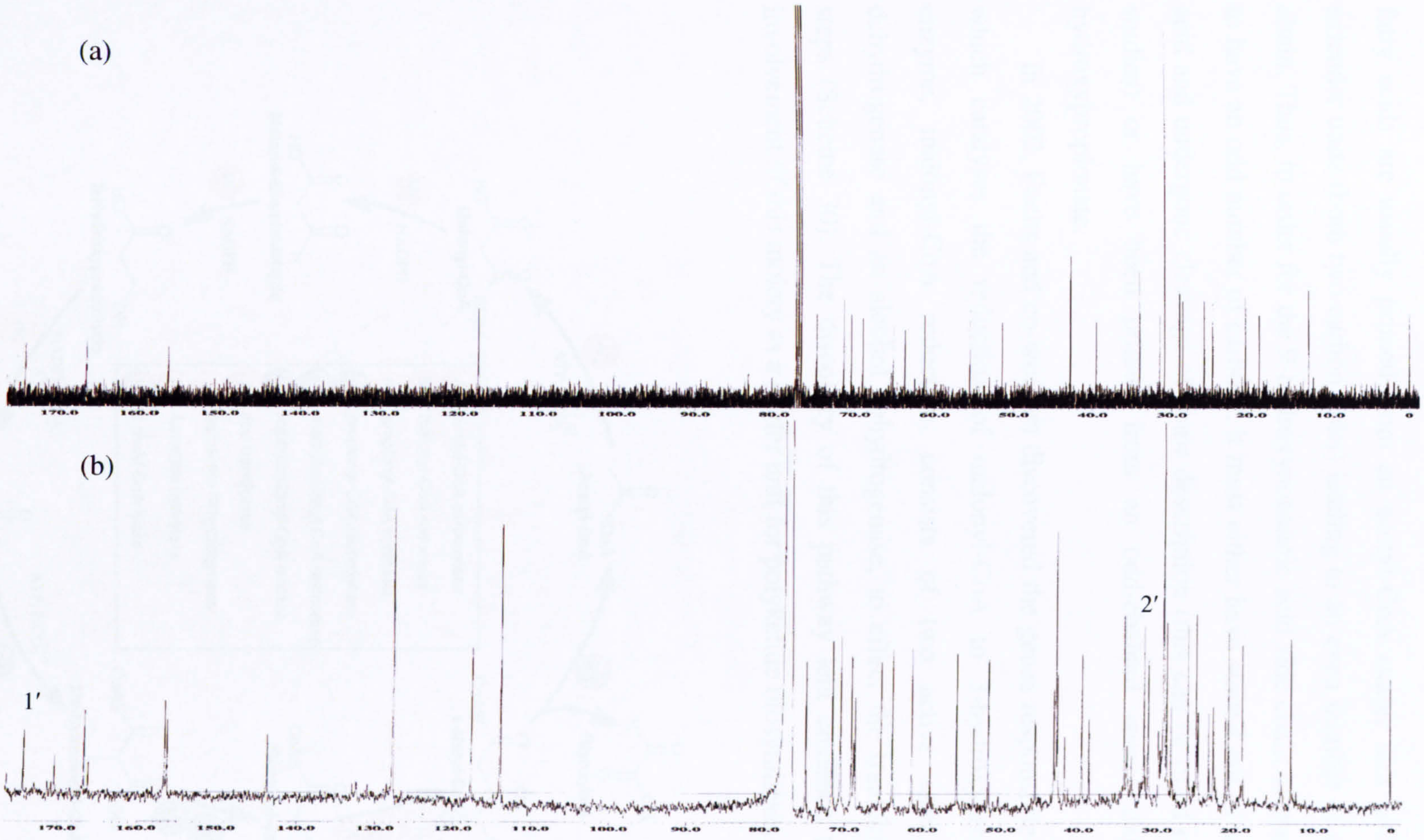
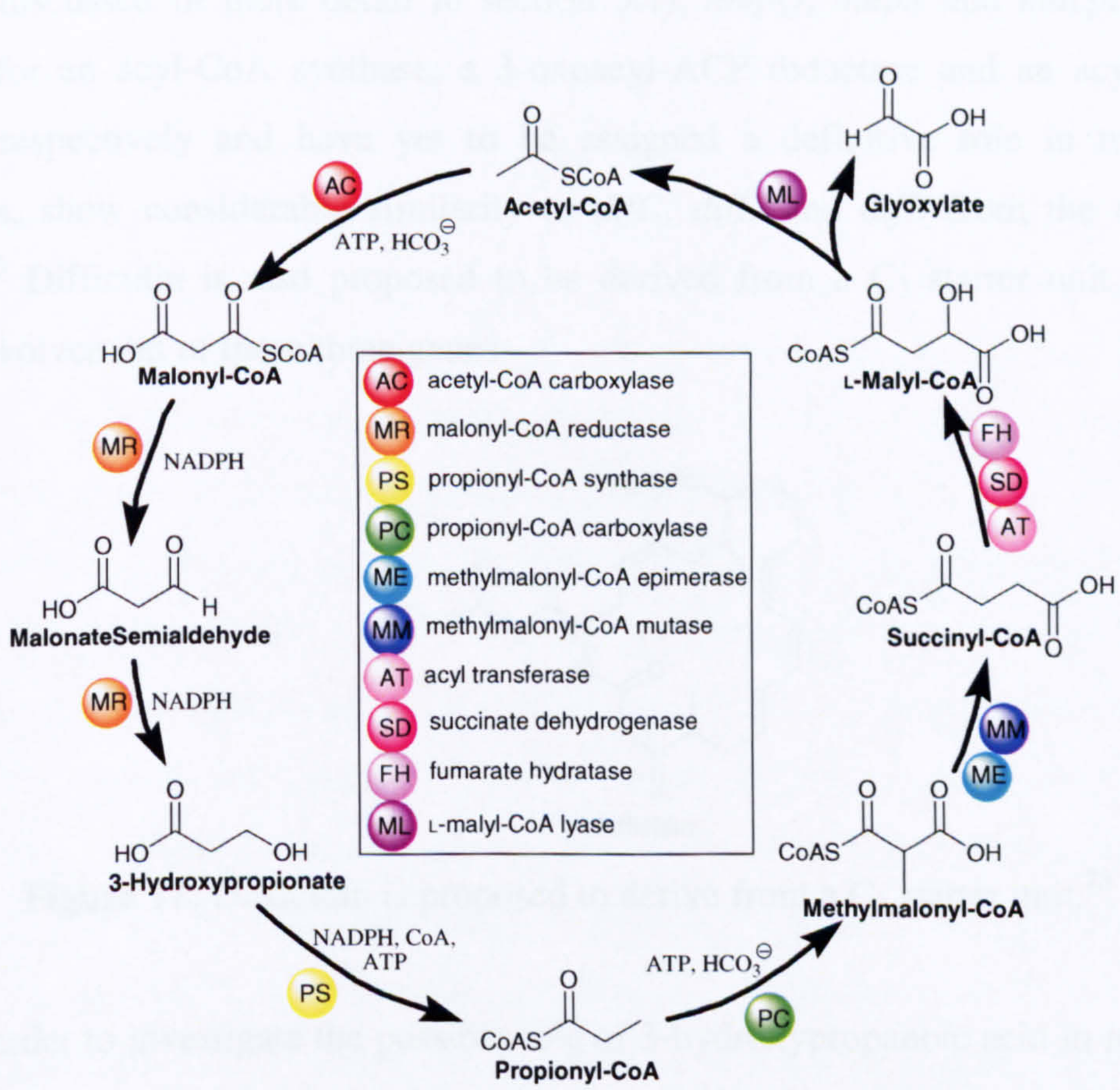


Figure 16: ^{13}C NMR spectra of (a) methyl pseudomonate standard and (b) methyl pseudomonate isolated after administration of thiol ester **1**.

2.5 Investigation of 3-Hydroxypropanoic Acid as a Polyketide Starter Unit

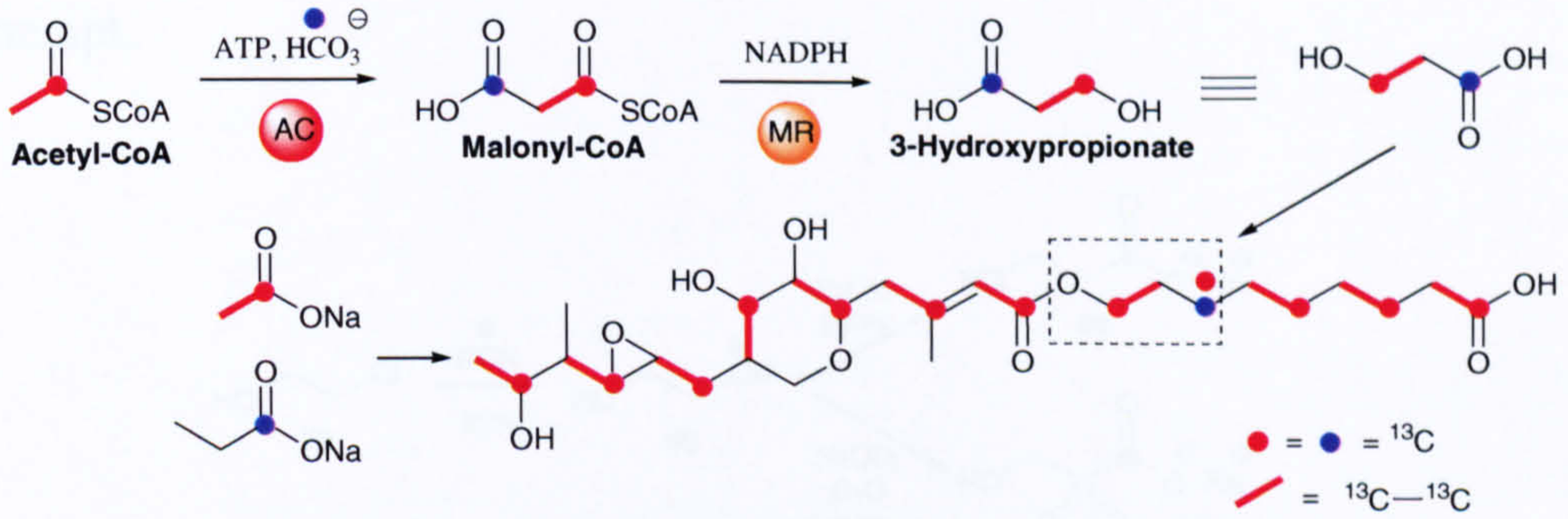
Odd-chained fatty acids are unusual in natural products. As discussed in section 1.1, fatty acids are usually primed from an acetyl-CoA starter unit and malonyl-CoA extender units (both two carbon units) leading to an even number of carbons in their chain. Thus, in order for the 9-hydroxynonanoic acid side chain of pseudomonic acid to have an odd number of carbons, it must either have started out as an even-chained acid and undergone cleavage of some description (this can be ruled out by labelling studies) or have been primed from an odd-chained starter unit such as 3-hydroxypropionate.

In 2002, Fuchs and co-workers discovered the genes responsible for an enzyme which catalyses the reduction of malonyl-CoA to 3-hydroxypropionate.[74] The enzyme, malonyl-CoA reductase, consists of two active sites, an aldehyde dehydrogenase and an alcohol dehydrogenase, to effect the transformation in two steps (Scheme 30). The discovery of this pathway lent credence to the possible involvement of this moiety as a starter unit for polyketide biosynthesis.



Scheme 30: Proposed 3-hydroxypropionate cycle.[74]

This mode of biosynthesis would also explain the unusual labelling pattern of the 9-hydroxynonanoic acid sidechain in which the C-8′–C-9′ unit deviates from the usual head-to-tail acetate incorporation generally observed for polyketide metabolites (Scheme 31) and would also explain why incorporation was only observed for C-1 of the propionate precursor, as the recyling of this entity to acetyl-CoA would result in the loss of any isotopic labels at the C-2 or C-3 positions.



Scheme 31: Unusual labelling pattern of 9-hydroxynonanoic acid side chain.[63]

Furthermore, homology testing shows that three of the mupirocin genes (which will be discussed in more detail in section 3.1), *mupQ*, *mupS* and *macpD*, which encode for an acyl-CoA synthase, a 3-oxoacyl-ACP reductase and an acyl carrier protein respectively and have yet to be assigned a definitive role in mupirocin synthesis, show considerable similarity to *difC*, *difD* and *difE* from the difficidin cluster.[75] Difficidin is also proposed to be derived from a C_3 starter unit with the likely involvement of these three genes.

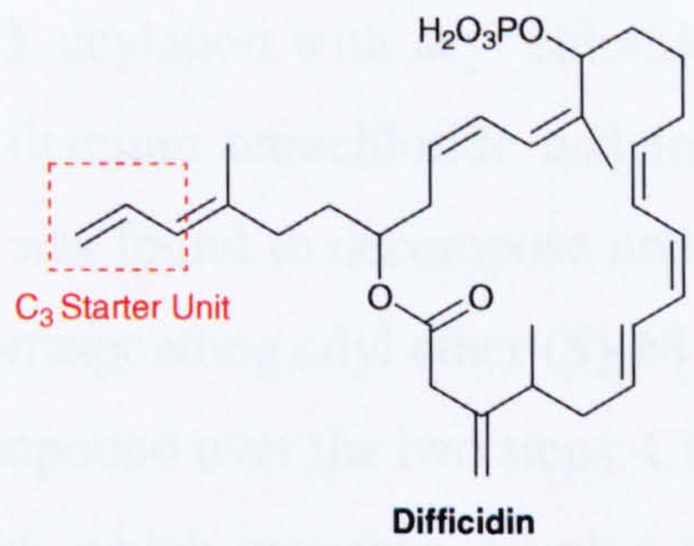
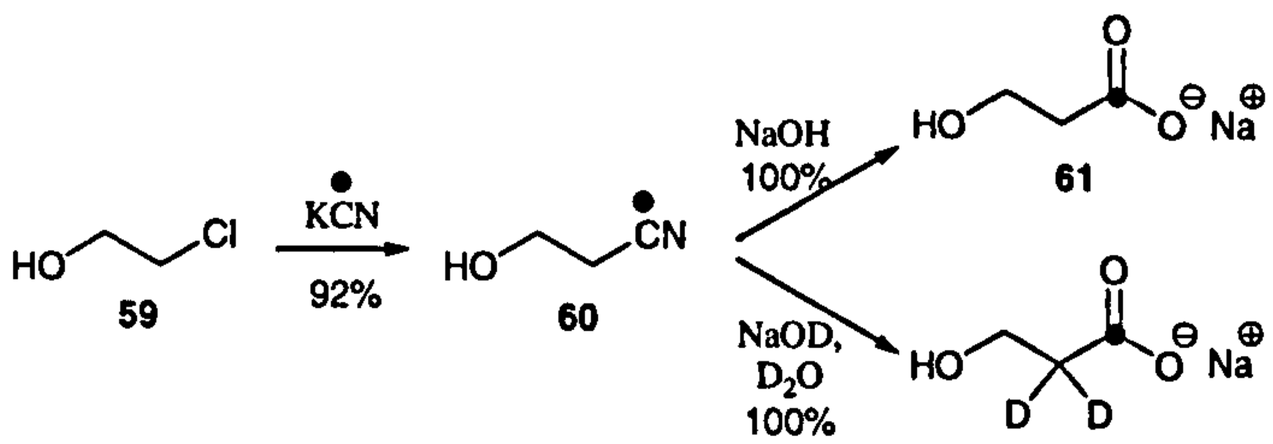


Figure 17: Difficidin is proposed to derive from a C_3 starter unit.[75]

In order to investigate the possible role of 3-hydroxypropanoic acid in mupirocin biosynthesis we aimed to prepare suitable isotopically labelled substrates and to conduct a feeding study with *Pseudomonas fluorescens*. Sugden[66] has previously

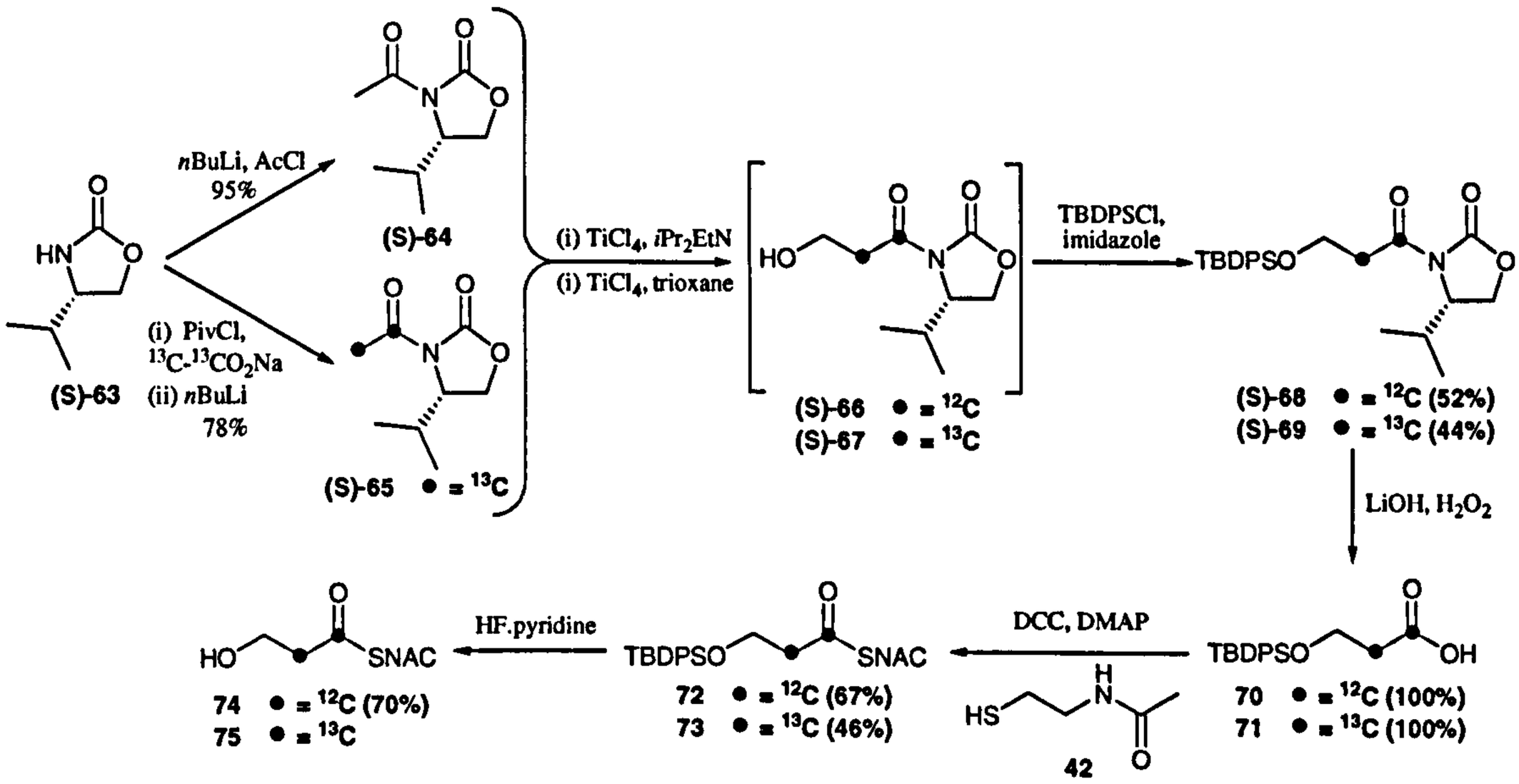
prepared sodium [1-^{13}C]-3-hydroxypropanoate **61** and sodium [1-^{13}C, 2-$^{2}H_2$]-3-hydroxypropanoate **62** (Scheme 32). Starting from 3-chloropropanol **59**, addition of [^{13}C]-potassium cyanide gave nitrile **60** which was then hydrolysed using either deuterated or protio sodium hydroxide to give the free acids **61** and **62** in quantitative yield. The two substrates were then fed in duplicate to *P. fluorescens*. Although initially the mono-labelled precursor **61** appeared to incorporate, the second feed did not substantiate this result. The tri-labelled propionate **62** failed to incorporate on either attempt.



Scheme 32: Sugden's synthesis of labelled 3-hydroxypropionoates **61** and **62**.[66]

Owing to the success of NAC thiol esters in feeding studies, we turned our attention towards the synthesis of [1,2-$^{13}C_2$]-3-hydroxypropanoic acid *N*-acetylcysteamine thiol ester **75**. It was hoped that not only would the thiol ester incorporate better than the free acid but also that the two vicinal ^{13}C labels would increase the sensitivity of the experiment. Watts has previously investigated the synthesis of **75** as shown in Scheme 33.[76] He optimised his route using unlabelled material, successfully preparing thiol ester **73** in 23% overall yield. Starting from Evans' oxazolidinone (*S*)-**63**, acylation with acyl chloride gave (*S*)-**64** in 95% yield. Hydroxymethylation using titanium tetrachloride and trioxane afforded the alcohol (*S*)-**66** which unfortunately was found to decompose under standard chromatography conditions. However, the corresponding silyl ether (*S*)-**68** could be isolated furnishing 52% yield of the desired compound over the two steps. Cleavage of the auxiliary gave acid **70** in quantitative yield, which was then coupled to *N*-acetylcysteamine **42** to give thiol ester **72**. Finally, deprotection of the silyl group afforded the desired thiol ester **74** in 70% yield. Adjusting the synthesis to incorporate vicinal ^{13}C labels *via* [1,2-$^{13}C_2$]-sodium acetate and pivaloyl chloride, Watts successfully prepared thiol

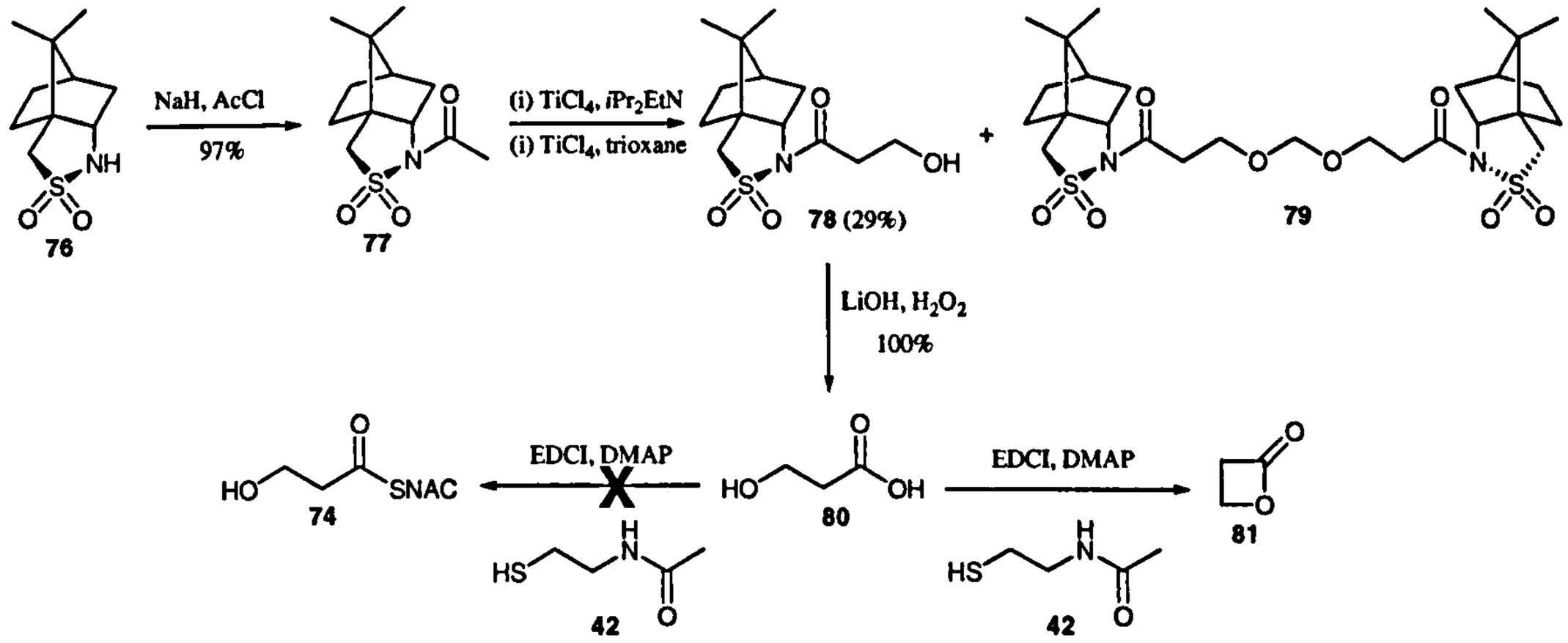
ester **73** in 16% overall yield but did not have enough material to effect the deprotection.



Scheme 33: Watts' preparation of thiol ester **74**.[76]

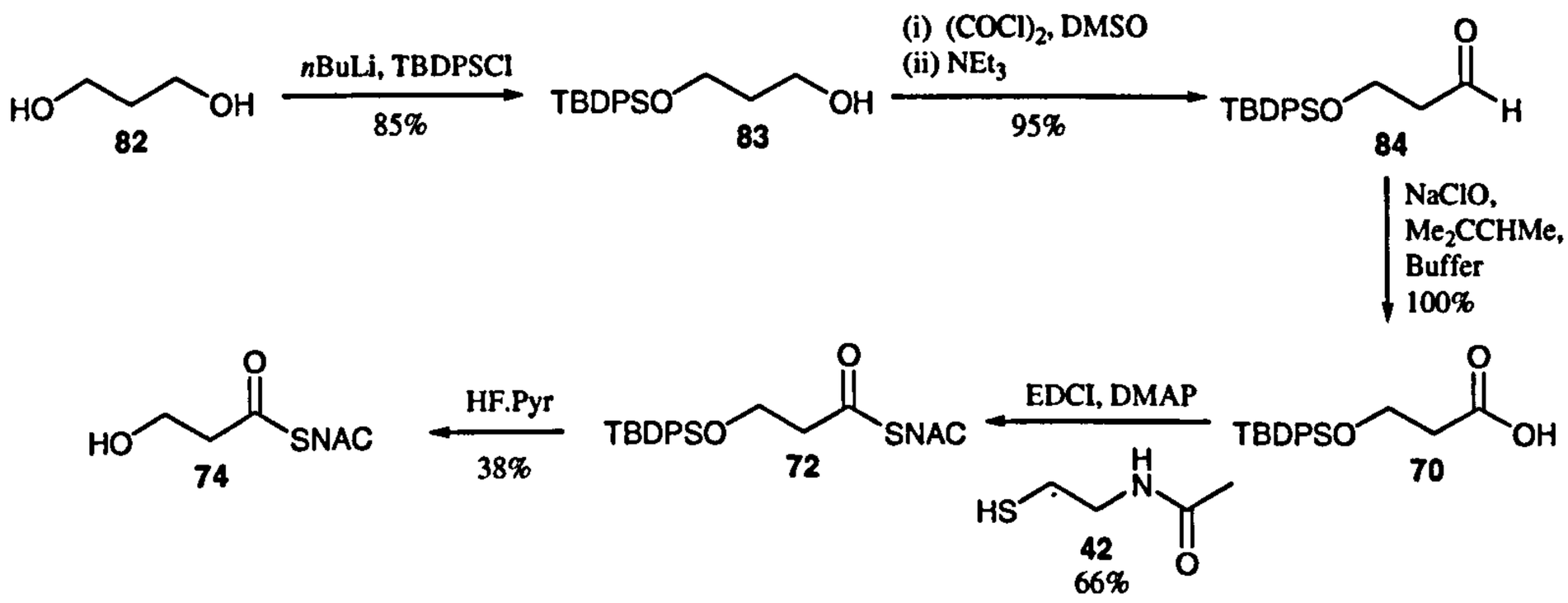
As this was a promising route, it was decided to try and further optimise the conditions. In particular, it was hoped to improve the yield of the hydroxymethylation step and also to attempt the synthesis without a protecting group. As the auxiliary was employed merely to avoid volatility and water solubility problems we investigated the use of a different auxiliary. Oppolzer's sultam **76** was chosen on the basis that it tends to produce crystalline solids which may be amenable to recrystallisation, thus offering an alternative purification to column chromatography. Acylation of auxiliary **76** gave acetylsultam **77** in 97% yield (Scheme 34). Hydroxymethylation afforded 29% of the desired alcohol **78** (this was the isolated yield though ^{1}H NMR analysis of mixed fractions showed that the real yield was probably closer to 46%) but interestingly dimer **79** (approximately 22% as determined by ^{1}H NMR analysis) was also observed. These two products were difficult to separate by column chromatography leading to very low yields for this step. Premixing the trioxane and titanium tetrachloride prior to addition did not aid matters, nor did adding 3% methanol (to aid formaldehyde monomer formation and prevent polymerisation).[77] Lowering the number of equivalents of trioxane decreased the yield further and attempts to protect the crude mixture as the TBDPS silyl ether also proved futile. Cleavage of the auxiliary with lithium hydroxide and hydrogen peroxide gave 3-hydroxypropanoic acid **80** in quantitative yield. However, attempts to couple acid **80** to *N*-acetylcysteamine **42**

were unsuccessful. None of the characteristic signals for thiol ester **74** were present in the NMR spectra of the crude product. In particular, a multiplet signal at 4.33-4.41 ppm in the ^{1}H NMR spectrum was too far upfield to be attributed to the 3-H_2 protons (or any of the reagent/by-product protons). It does however, compare well with the 3-H_2 signal of β-lactone **81** which comes at 4.28 ppm[78] and so this seems the most plausible explanation.



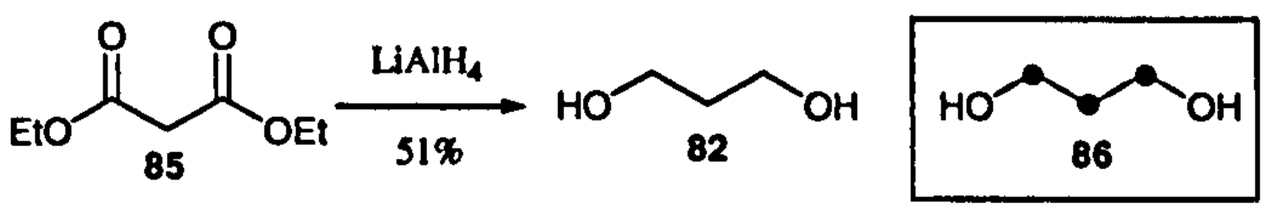
Scheme 34: Towards the synthesis of thiol ester **74**.

Reverting to Watts' choice of auxiliary, Evans' oxazolidinone **63**, did nothing to improve matters and so it was decided to develop a new route which would avoid installing a one-carbon unit (Scheme 35). Mono-protection of diol **82** gave silyl ether **83** in 85% yield. Swern oxidation afforded aldehyde **84** in 95% yield which then underwent a Pinnick type oxidation with sodium chlorite to furnish acid **70** in quantitative yield. This was then coupled to *N*-acetylcysteamine **42** to give thiol ester **72** and deprotected using hydrogen fluoride pyridine complex to give the desired thiol ester **74** in 38% yield.



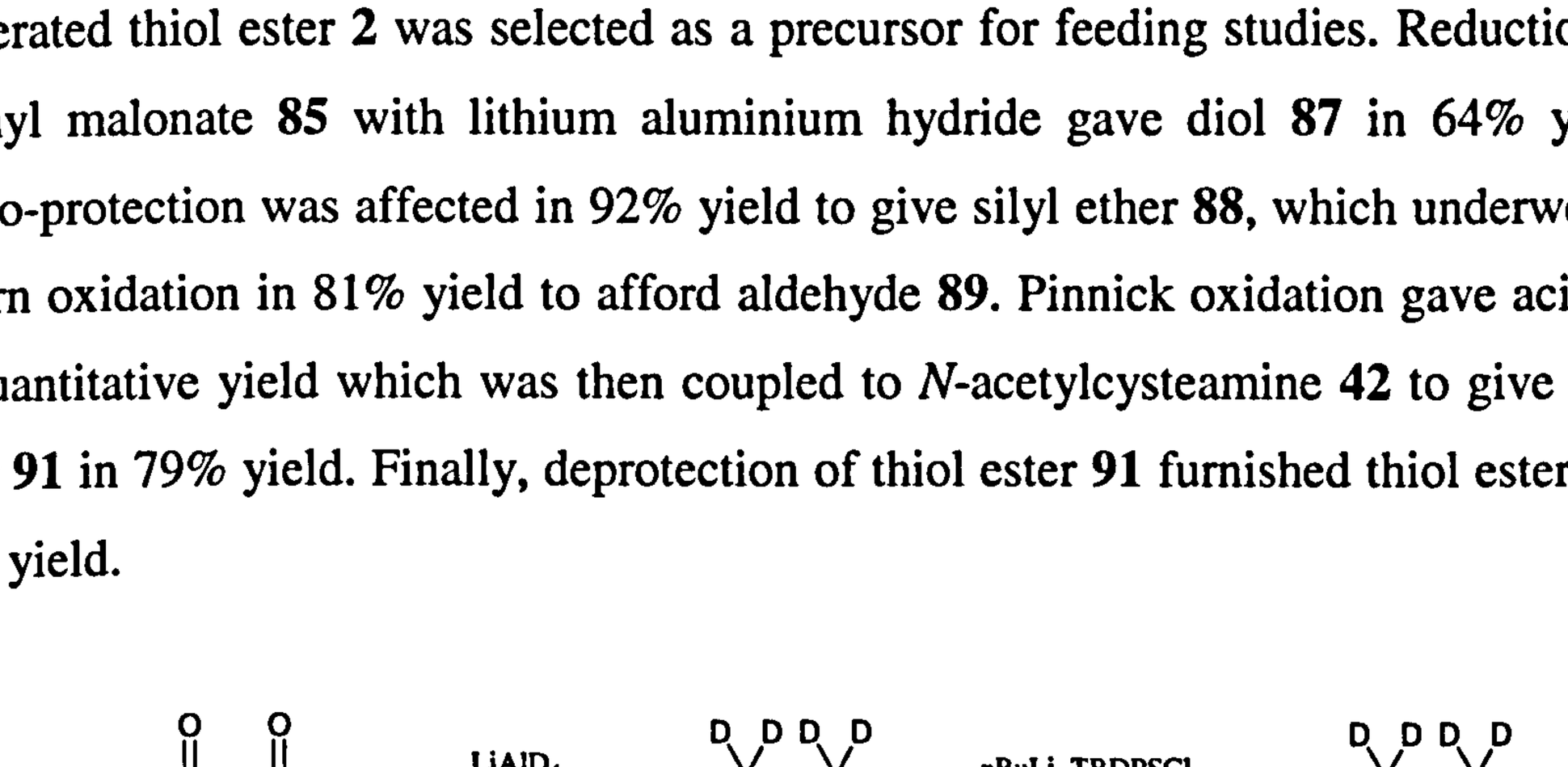
Scheme 35: Revised route to thiol ester **74**.

As all three carbons in the final thiol ester come from the starting diol **82**, this must be the source for any isotopic labels. Due to the symmetrical nature of the starting material and the desire for two vicinal ^{13}C labels, the only commercially available option is [$^{13}C_3$]-1,3-propanediol **86**. As this is extremely expensive we opted to investigate its synthesis. Reduction of diethyl malonate **85** with lithium aluminium hydride gave diol **82** in 51% yield (Scheme 36). However, although comparable to literature yields for this reaction (the highest yield in the literature is 63%[79]), overall this route was deemed too inefficient for expensive ^{13}C substrates.



Scheme 36: Preparation of diol **82** from diethyl malonate **85**.

The new route was however ideal for deuterium incorporation. Lithium aluminium deuteride is a relatively cheap source of isotopic label and thus the deuterated thiol ester **2** was selected as a precursor for feeding studies. Reduction of diethyl malonate **85** with lithium aluminium hydride gave diol **87** in 64% yield. Mono-protection was affected in 92% yield to give silyl ether **88**, which underwent a Swern oxidation in 81% yield to afford aldehyde **89**. Pinnick oxidation gave acid **90** in quantitative yield which was then coupled to *N*-acetylcysteamine **42** to give thiol ester **91** in 79% yield. Finally, deprotection of thiol ester **91** furnished thiol ester **2** in 38% yield.



Scheme 37: Preparation of thiol ester **2**.

Thiol ester **2** was fed to *P. fluorescens* in duplicate (as described in section 2.2). Unfortunately, no pseudomonic acid was isolated on either attempt nor from the control flasks and so the only conclusion that can be drawn from these experiments is that the bacteria had stopped producing. Due to time constraints this experiment could not be repeated with a fresh batch of *P. fluorescens*.

2.6 Conclusions and Further Work

[1,2-$^{13}C_2$]-9-Hydroxynonanoic acid NAC thiol ester **1** was prepared in 5 steps and 39% overall yield. Once a growth production study had been conducted, thiol ester **1** was fed to *P. fluorescens* but did not show any incorporation of ^{13}C into the isolated pseudomonic acid. Taken in conjunction with the results of Sugden,[66] and the recent truncated metabolites **57** and **58** isolated by Wu (J. Wu, unpublished results), this may imply that the 9-hydroxynonanoic acid chain is not preformed before esterification to the monic acid fragment but is constructed *in situ* once this union has occured. However, care must be taken in interpreting these results as there are many reasons why feeding experiments might be unsuccessful.

[3,3-$^{2}H_2$]-3-Hydroxy propanoic acid NAC thiol ester **2** was also prepared and fed to *P. fluorescens*. Unfortunately, both feeding studies with thiol ester **2**, including the control flasks, failed to produce any pseudomonic acid. The only conclusion that can be drawn from these experiments is that *P. fluorescens* had stopped producing. Martin[70] and Sugden[66] have both previously reported problems with inconsistent production of pseudomonic acid. This experiment will need to be repeated with the remaining material when a fresh batch of *P. fluorescens* is available.

Chapter 3

The Pseudomonic Acids: Synthesis of Mutant Metabolites

3.1 Investigation of the Mupirocin Gene Cluster

In 2003 Thomas and co-workers sequenced the mupirocin gene cluster and assigned putative enzymatic functions to many of the genes/domains identified, based on their similarity to other polyketide and fatty acid synthases (Figure 18 and Table 1).[80]

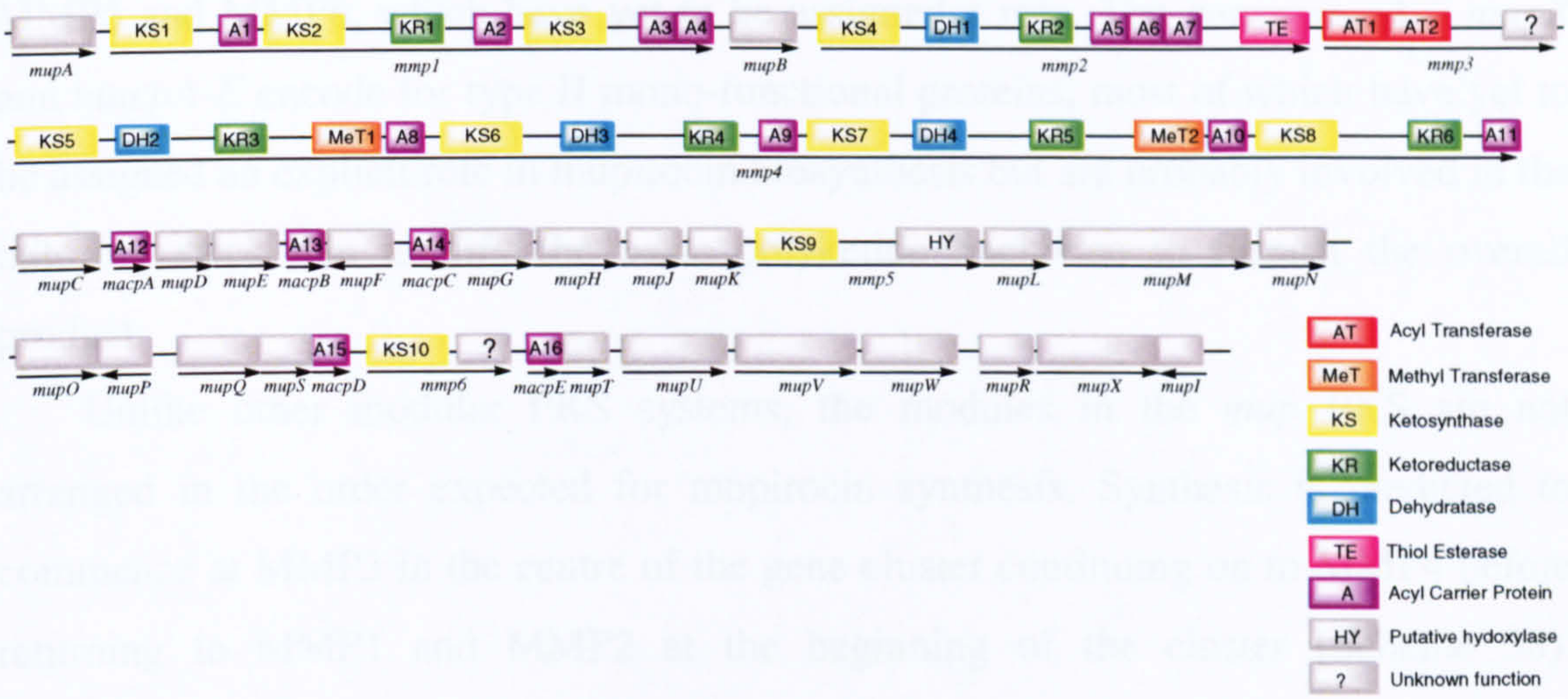


Figure 18: The mupirocin gene cluster.[80]

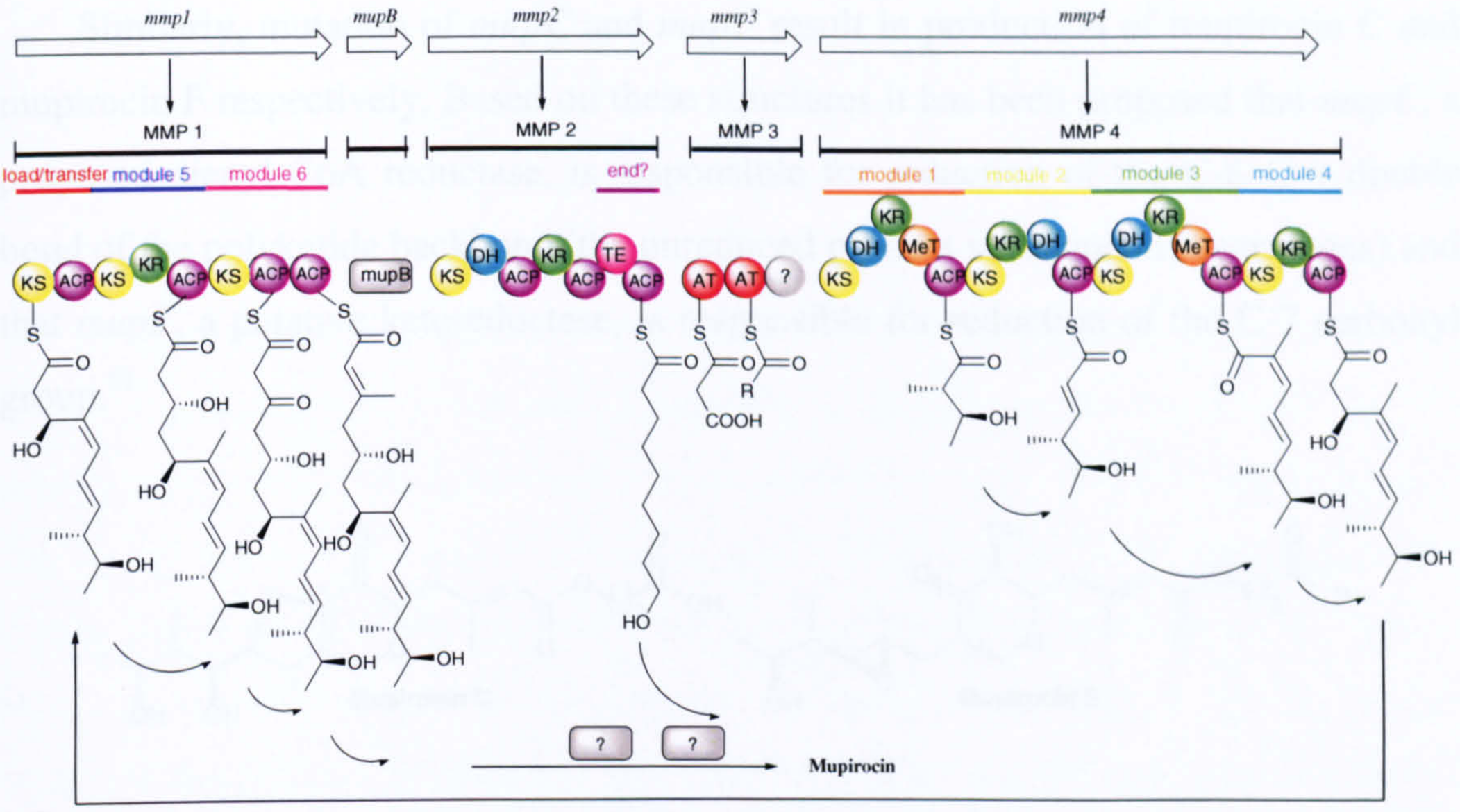
Gene	Putative Function	Gene	Putative Function
mupA	$FMNH_2$-dependent oxygenase	*mupN*	regulatory DNA binding protein
mupB	3-oxoacyl-ACP synthase	*mupO*	cytochrome P450
mupC	NADH/NADPH oxidoreductase	*mupP*	function unknown
mupD	3-oxoacyl-ACP reductase	*mupQ*	acyl-CoA synthase
mupE	enoyl reductase	*mupS*	3-oxoacyl-ACP reductase
mupF	ketoreductase	*mupT*	ferredoxin dioxygenase
mupG	3-oxoacyl-ACP synthase I	*mupU*	acyl-CoA synthase
mupH	HMG-CoA-synthase	*mupV*	oxidoreductase
mupJ	enoyl-CoA-hydratase	*mup*	dioxygenase
mupK	enoyl-CoA-hydratase	*mupR*	N-AHL-responsive transcriptional activator
mupL	hydrolase	*mupX*	amidase/hydrolase
mupM	isoleucyl-tRNA synthase	*mupI*	N-AHL synthase

Table 1: Putative functions assigned to mupirocin type II genes by Thomas and co-workers based on homology testing.[80]

The mupirocin PKS is unusual in that it contains elements of both type I and type II PKS structure and is both iterative and modular in places.[80] The large multifunctional mupirocin (*mmp*) genes encode for the modular type I proteins - MMP 1, MMP3 and MMP4 proposed to be responsible for the synthesis of the monic acid precursor, MMP2, an iterative type I protein thought to be responsible (in conjunction with *mupE*) for the production of the 9-hydroxynonanoic acid entity and MMP5 and MMP6, which have yet to be assigned a role. The genes *mupA – mupX* and *macpA-E* encode for type II mono-functional proteins, most of which have yet to be assigned an explicit role in mupirocin biosynthesis but are probably involved in the tailoring steps that modify the basic polyketide backbone to furnish the overall product.

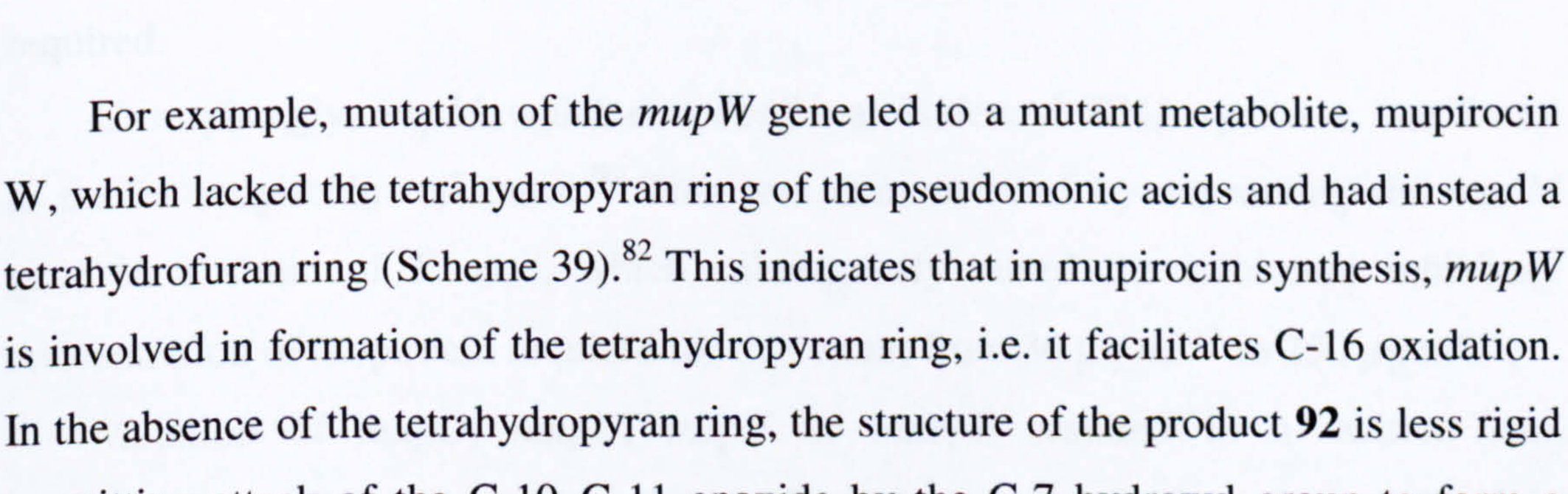
Unlike other modular PKS systems, the modules in the *mup* PKS are not arranged in the order expected for mupirocin synthesis. Synthesis is predicted to commence at MMP3 in the centre of the gene cluster continuing on to MMP4 before returning to MMP1 and MMP2 at the beginning of the cluster (Scheme 38). Positioning of the thiol esterase domain in the centre of the gene cluster rather than at the end is highly unusual, leading to suggestions that perhaps this domain catalyses release of the 9-hydroxynonanoic acid entity rather than the fully synthesised mupirocin or performs the condensation step which unites the two moieties. A further unusual characteristic is the lack of acyl transferase domains within each of the condensation modules. How exactly the extender units are delivered to the growing chain is still a mystery. A deeper understanding of the unique features of the mupirocin polyketide synthase may therefore help to explain not only the mechanism by which mupirocin is synthesised but also the mechanism by which many other complex polyketide metabolites are furnished.

To date, several gene deletions have been carried out on the mupirocin gene cluster. Knock out experiments on the ACPs, KS1, KR1, TE, AT1, AT2, MeT1 domains, the multifunctional genes *mmpE* and *mmpF*, and the *mupC*, *mupD*, *mupE*, *mupF*, *mupG*, *mupH*, *mupI*, *mupJ*, *mupK*, *mupL*, *mupM*, *mupN*, *mupO*, *mupQ*, *mupR*, *mupS*, *mupT*, *mupU*, *mupV*, *mupW*, *mupX*, *macpA*, *macpB*, *macpC*, *macpD* and *macpE* monofunctional genes all resulted in a loss/reduction of pseudomonic acid production and in some cases the appearance of new products.[81]



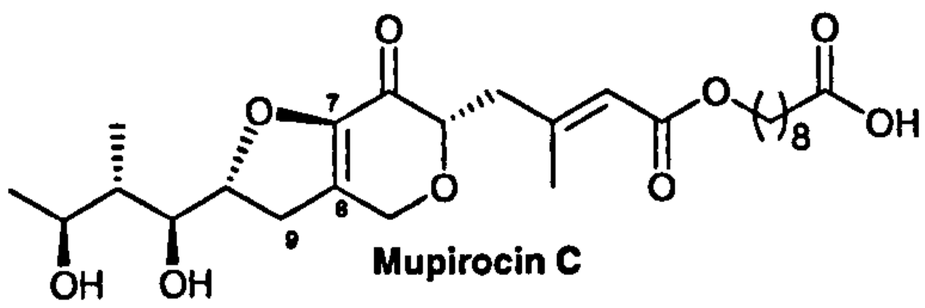
Scheme 38: Proposed domain organisation of the mupirocin polyketide synthase and putative biosynthetic intermediates.[80]

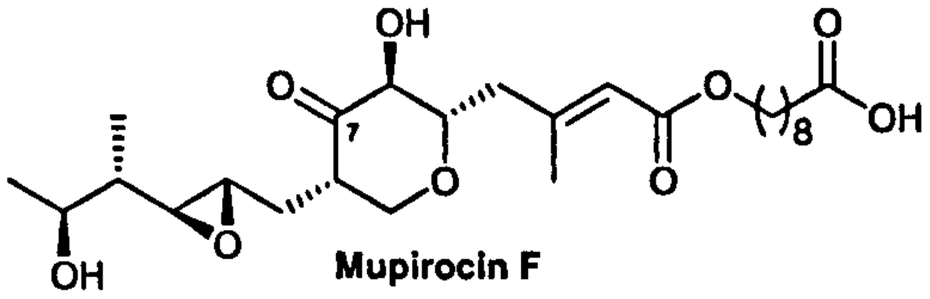
For example, mutation of the *mupW* gene led to a mutant metabolite, mupirocin W, which lacked the tetrahydropyran ring of the pseudomonic acids and had instead a tetrahydrofuran ring (Scheme 39).[82] This indicates that in mupirocin synthesis, *mupW* is involved in formation of the tetrahydropyran ring, i.e. it facilitates C-16 oxidation. In the absence of the tetrahydropyran ring, the structure of the product **92** is less rigid permitting attack of the C-10–C-11 epoxide by the C-7 hydroxyl group to form a tetrahydrofuran ring. Whether this transformation occurs *in situ* or as a consequence of the acidic work-up has yet to be determined.



Scheme 39: Mutation of *mupW* results in mupirocin W.[82]

Similarly, mutation of *mupC* and *mupF* result in production of mupirocin C and mupirocin F respectively. Based on these structures it has been proposed that *mupC*, a proposed dienyl-CoA reductase, is responsible for reduction of the C-8–C-9 double bond of the polyketide backbone (the unreduced product subsequently rearranges) and that *mupF*, a putative ketoreductase, is responsible for reduction of the C-7 carbonyl group.[81]

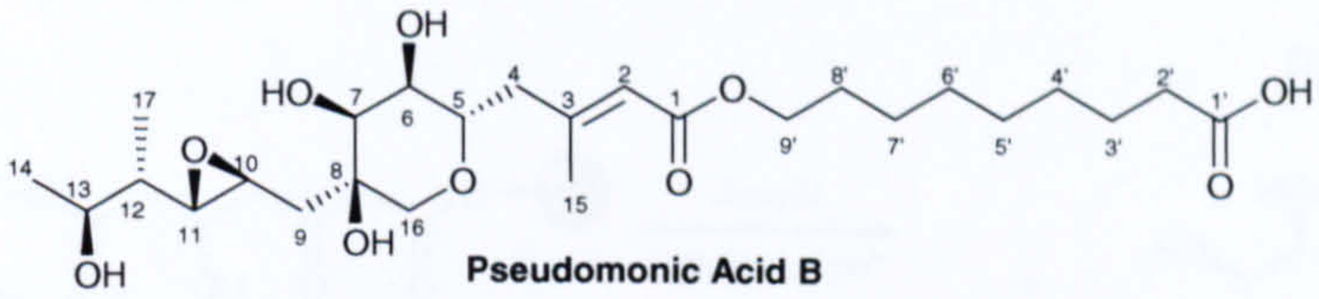




MupI and *mupR* (which are named out of sequence) showed significant identity with quorum sensing regulatory proteins such as those encoded by *LasI* and *LasR* from *Pseudomonas aeruginosa* and *LuxI* and *LuxR* from *Vibrio fischeri*.[83] MupI was found to produce a diffusible inducer molecule which activates the *mup* promoter region located upstream of *mupA*, effectively switching on mupirocin biosynthesis. MupR is likely to act as a regulator, boosting and suppressing *mupI* expression as required.

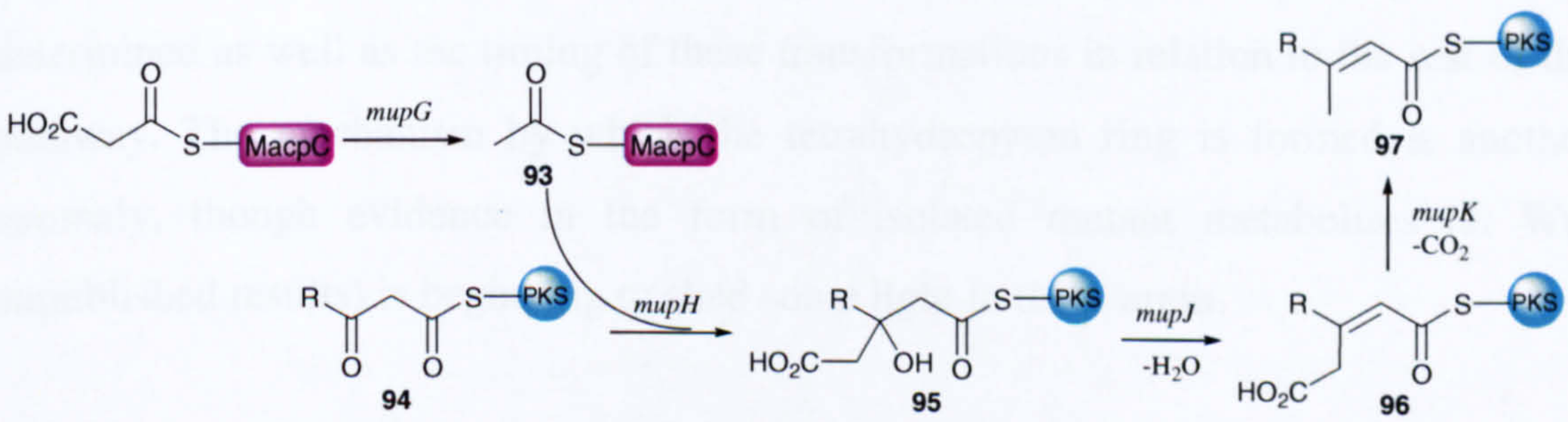
Unsurpisingly, *mupM*, which encodes for an isoleucyl tRNA synthase, was found to confer mupirocin resistance.[80] This was demonstrated by expressing the *mupM* gene in a strain of *E. coli*, which subsequently raised the minimum inhibitory concentration of mupirocin required for that strain from 30 $\mu g\ mL^{-1}$ to 250 $\mu g\ mL^{-1}$.

Mutation of *mupO*, *mupU*, *mupV* or *macpE* resulted in a switch from pseudomonic acid A to pseudomonic acid B production.[84] Pseudomonic acid B might be expected to be derived from pseudomonic acid A by a simple hydroxylation at the C-8 position. However, the accumulation of pseudomonic acid B at the expense of pseudomonic acid A, seems to suggest that it is either a precursor to pseudomonic acid A or a shunt product of the pathway. The latter is in agreement with the findings of Mantle *et al.*,[85] who found that the relative patterns of radioactivity resulting from feeding studies with [1-^{14}C]-acetate and L-[methyl-^{14}C]-methionine at early, mid and late stages of fermentation, were inconsistent with the assumption that pseudomonic acid B results from oxidation of pseudomonic acid A or that pseudomonic acid A results from reduction of pseudomonic acid B.



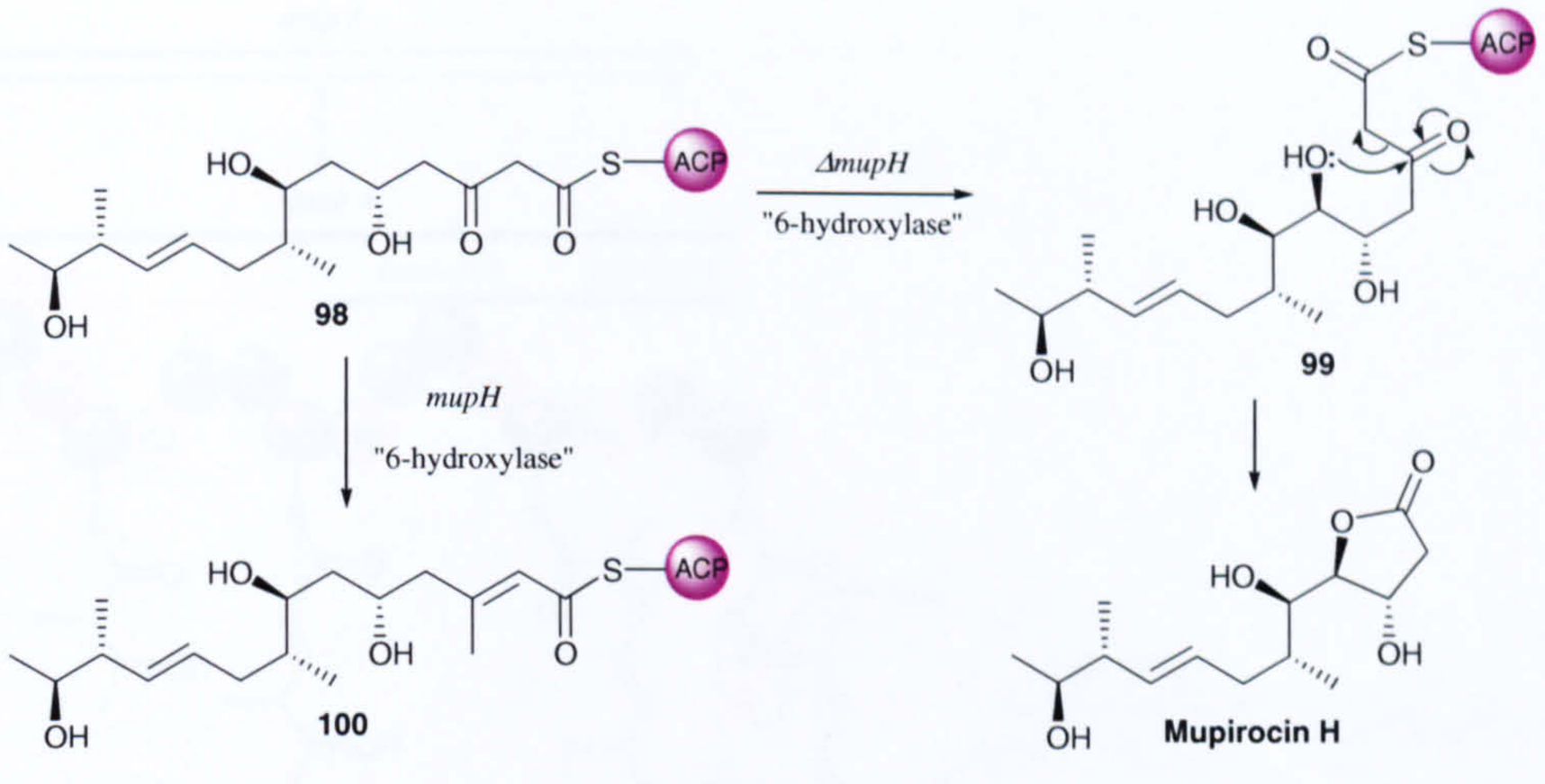
Pseudomonic Acid B

Mupirocin H, initially isolated by mutation of the *mupH* gene but also a product of *macpC*, *mupG*, *mupJ* and *mupK* mutation is thought to be responsible for the acylation step in module 6 which in conjunction with *macpC*, *mupG*, *mupJ* and *mupK* introduces the 15-CH_3 moiety (Scheme 40).[86] MupH is proposed to catalyse the condensation of thiol ester **94** with acetate **93**. The latter is produced by MupG catalysed decarboxylation and is delivered to the PKS by MacpC. Finally, MupJ catalysed dehydration of glutarate analogue **95** to give acid **96** and MupK catalysed decarboxylation furnishes β-methyl-α,β-unsaturated thiol ester **97**.



Scheme 40: Proposed mechanism for introduction of the 15-methyl group.[86]

Mutagenesis of *mupH*, which encodes a HMG-CoA-synthase, blocks this step (Scheme 41).[87] Thus, hydroxylation of **98** at C-6 to give **99**, allows an intramolecular attack of the 6-hydroxyl onto the β-carbonyl of the thiol ester and a subsequent retro-aldol reaction affording mupirocin H instead of the proposed product of module 6, thiol ester **100**. That all five mutations result in the same product may be due to a knock on effect.[86] It is proposed that mutation of any of the individual functionalities impairs the overall progess of the enzyme bound metabolites along the biosynthetic assembly line, allowing time for 6-hydroxylation and cyclisation to occur.

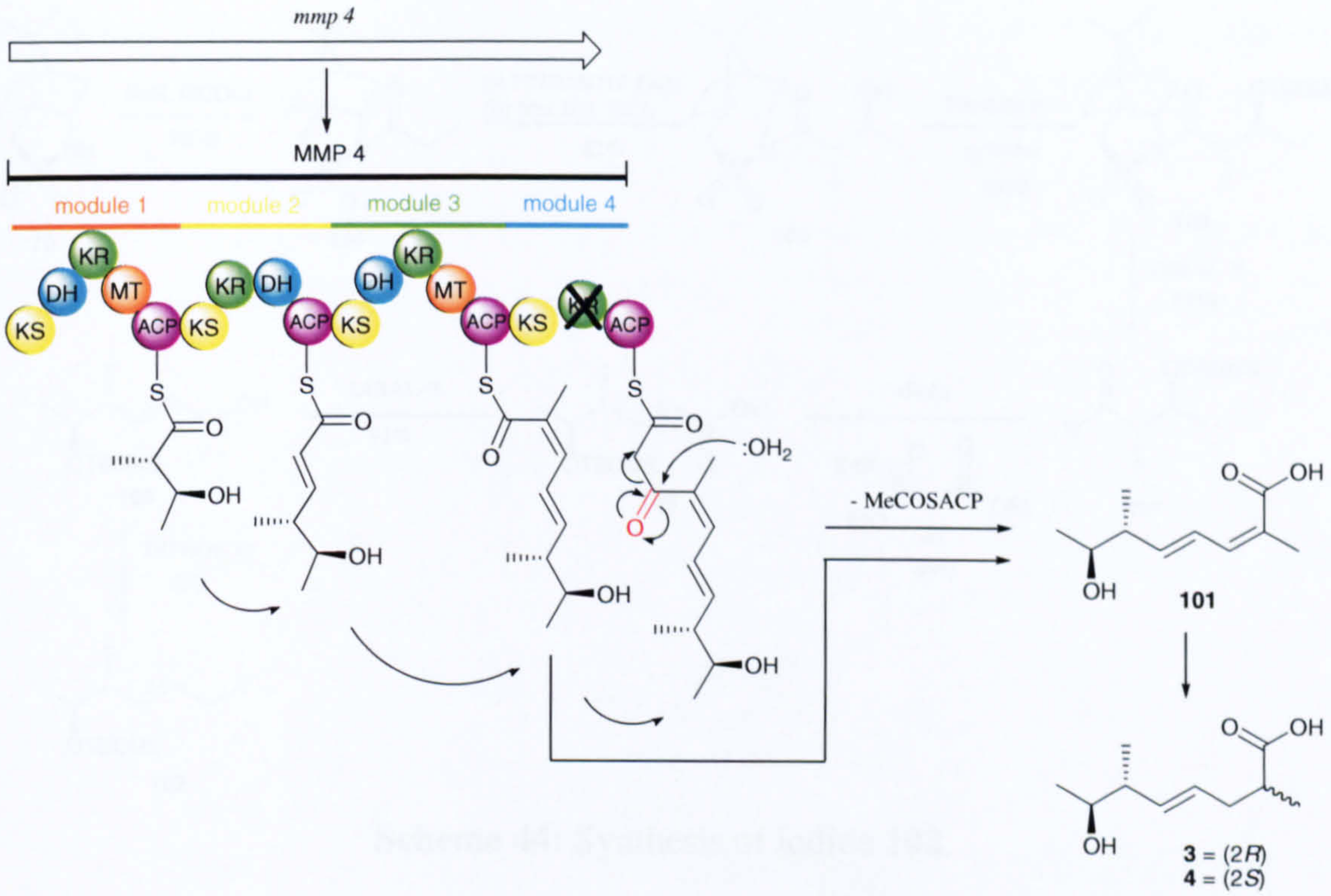


Scheme 41: Disruption of *mupH* blocks acylation, leading to mupirocin H.[87]

However, although much has been ascertained about the mupirocin biosynthetic pathway to date, there are still many unanswered questions. In particular, the genes responsible for C-10–C-11 epoxidation and 6-hydroxylation have yet to be determined as well as the timing of these transformations in relation to the rest of the pathway. The mechanism by which the tetrahydropyran ring is formed is another anomaly, though evidence in the form of isolated mutant metabolites (J. Wu, unpublished results) is beginning to shed some light in these areas.

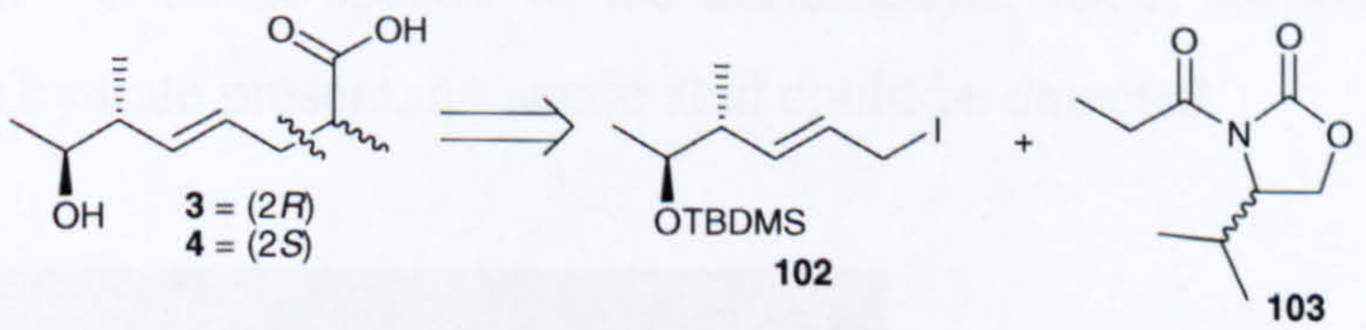
3.2 Synthesis of Mupiric Acid 3 and the C-2 Epimer 4

Mutation of the KR6 domain of the mupirocin gene cluster (and more recently mutation of the *macpC*, *mupB*, *mupG*, *mupJ*, *mupK*, *mupL*, *mupQ*, *mupS*, *mmpF* and *ACP5* (J. Wu, unpublished results)) also led to the production of a novel metabolite, mupiric acid **3** (Scheme 42). Like mupirocin H, mupiric acid **3** is thought to form as a result of congestion in the metabolic pathway, diverging either by hydrolysis of the module 3 product or by nucleophilic attack of the β-carbonyl (resulting from deactivation of the KR6 domain) of the module 4 thiol ester and a subsequent retro-aldol reaction (analogous to the mechanism proposed for mupirocin H formation). The resulting diene **101** then undergoes olefin reduction at the C-2–C-3 position to give mupiric acid **3**. The structure of mupiric acid **3** was tentatively assigned by comparison with pseudomonic acid.[86] However, in order to clarify that all stereochemical assignments were correct, a synthetic standard of acid **3** and its C-2 epimer **4** were required.



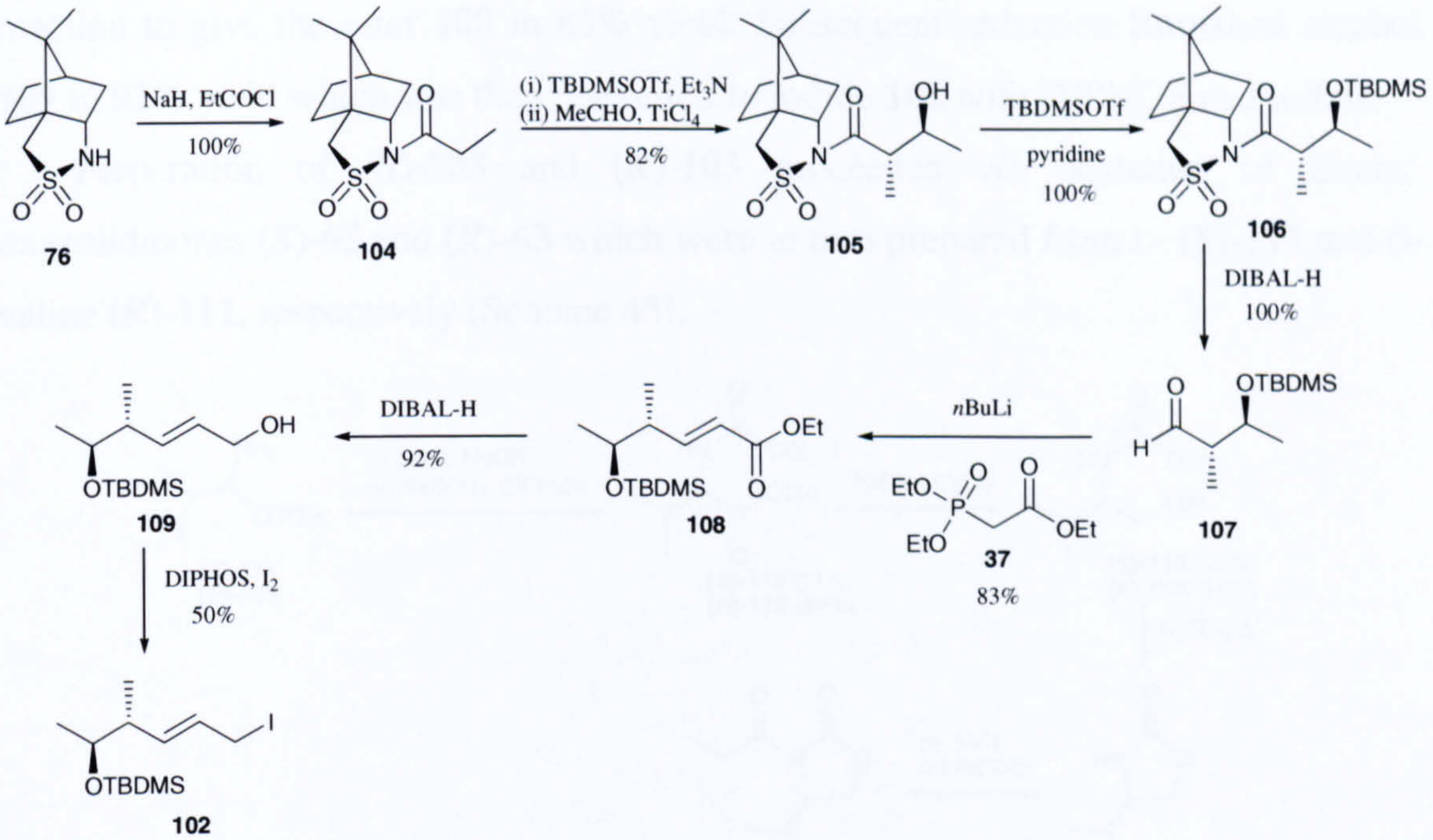
Scheme 42: Proposed biosynthesis of the truncated metabolite mupiric acid **3**.

Initial retrosynthetic analysis led us to iodide **102**, which had previously been prepared within our group by McKay[61,88] and the known oxazolidinone **103**[89] (Scheme 43). Furthermore, by varying the enantiomer of Evans' auxiliary used, it would be possible to gain access to both C-2 epimers.



Scheme 43: Retrosynthetic analysis of acids **3** and **4**.

Iodide **102** was prepared following the synthesis of McKay[88] (Scheme 44). Starting from Oppolzer's auxiliary **76**, acylation using propionyl chloride furnished propionylsultam **104** in quantitative yield. This was subsequently treated with *tert*-butyldimethylsilyl triflate and triethylamine, followed by titanium tetrachloride and acetaldehyde to yield alcohol **105** in 82% yield.



Scheme 44: Synthesis of iodide **102**.

This aldol step was particularly troublesome, requiring impeccably dry conditions for successful formation of the silyl enol ether moiety. Whilst trying to optimise the reaction conditions, a substantial quantity of a by-product was observed whenever an excess of acetaldehyde (relative to titanium (IV) chloride) was used. Spectral analysis confirmed the structure as acetate **110** and X-ray crystallography confirmed the stereochemistry. We were unable to determine exactly how this product formed. ^{1}H and ^{13}C NMR spectra of the acetaldehyde used, showed that although there was some hydrate present, no acetic acid could be detected.

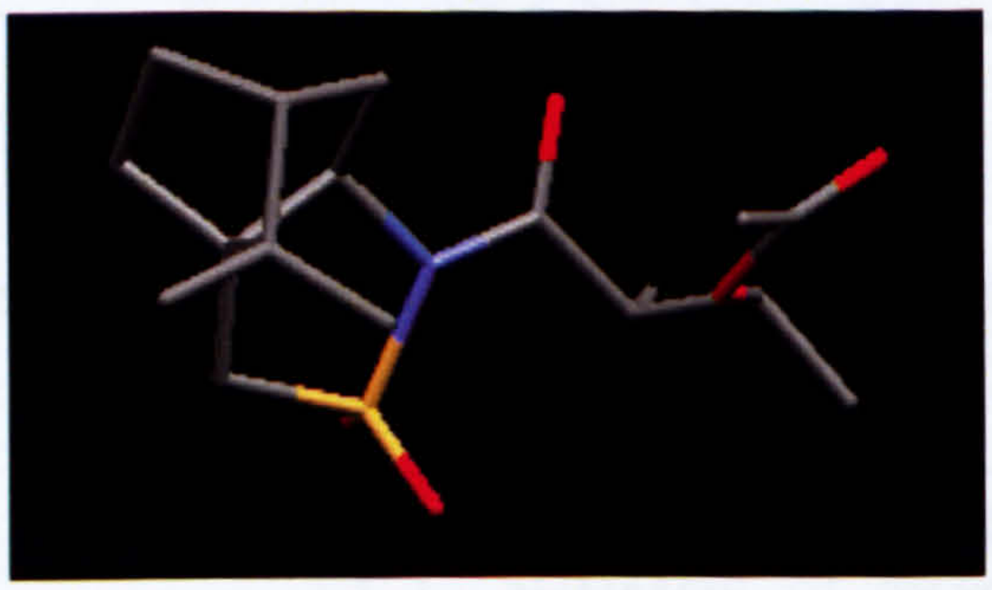
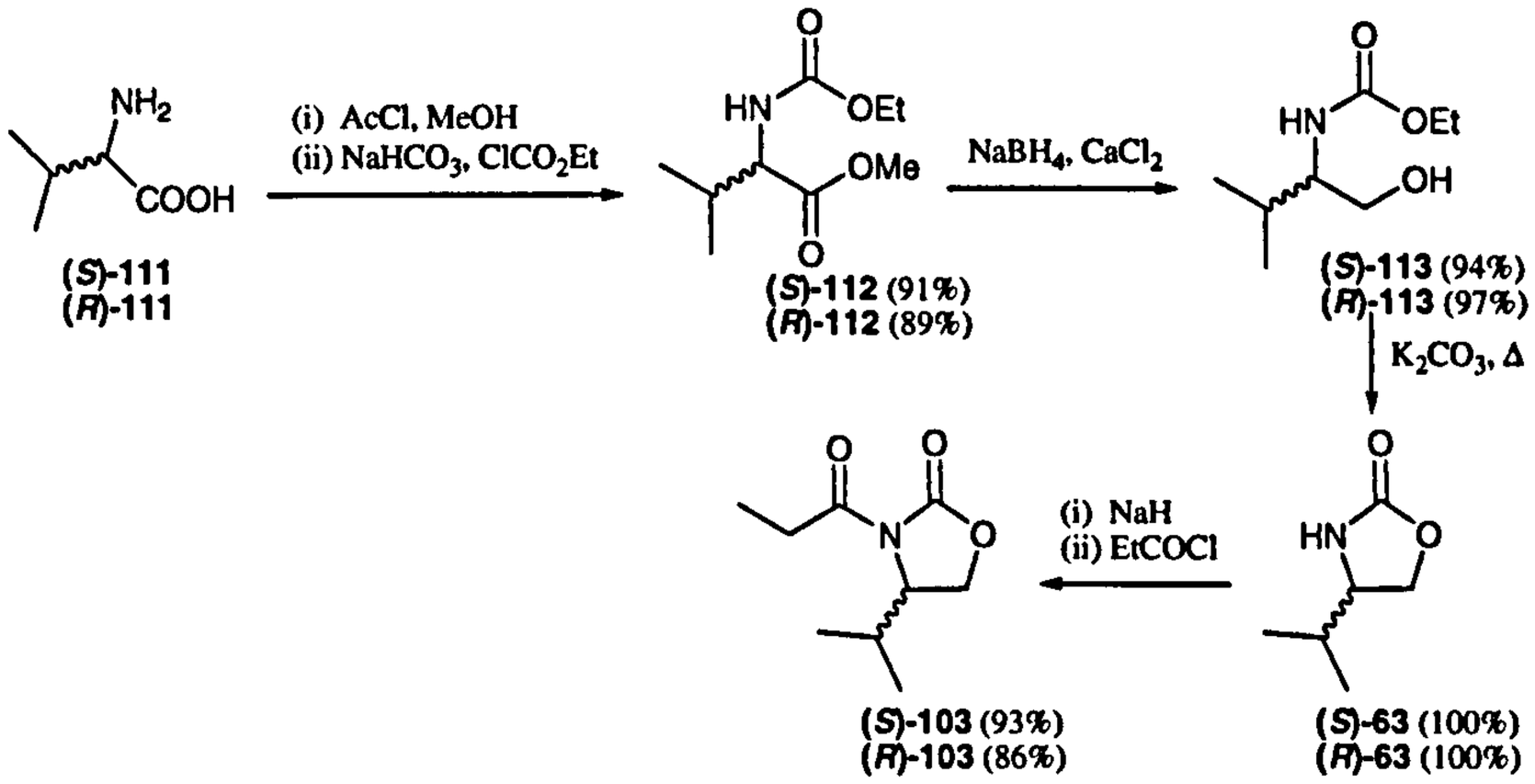


Figure 19: X-ray structure of acetate by-product **110**.

Subsequent protection of the 3′-hydroxyl group with *tert*-butyldimethylsilyl gave silyl ether **106** in quantitative yield. Removal of the auxiliary with DIBAL-H afforded aldehyde **107**, which was then chain extended by Horner-Wadsworth-Emmons

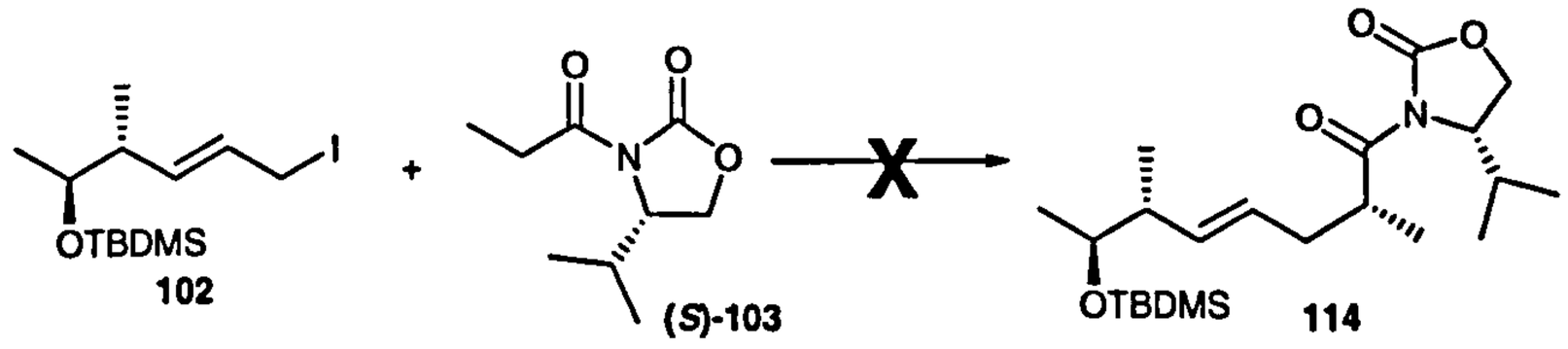
reaction to give the ester **108** in 83% yield. Subsequent reduction furnished alcohol **109** in 92% yield which was then converted to iodide **102** with DIPHOS and iodine.

Preparation of **(*S*)-103** and **(*R*)-103** proceeded *via* acylation of Evans' oxazolidinones **(*S*)-63** and **(*R*)-63** which were in turn prepared from L- **(*S*)-111** and D-valine **(*R*)-111**, respectively (Scheme 45).



Scheme 45: Preparation of alkylation substrates.

With both reactants in hand, we turned our attention towards the alkylation of **(*S*)-103** with iodide **102** (Scheme 46). Unfortunately however, all attempts to effect this union proved futile, as none of the desired product **114** was observed. Table 2 summarises the various conditions investigated. In each case, no reaction was observed by T.L.C. at -78 °C and gradual warming to room temperature simply resulted in decomposition of **(*S*)-103**.

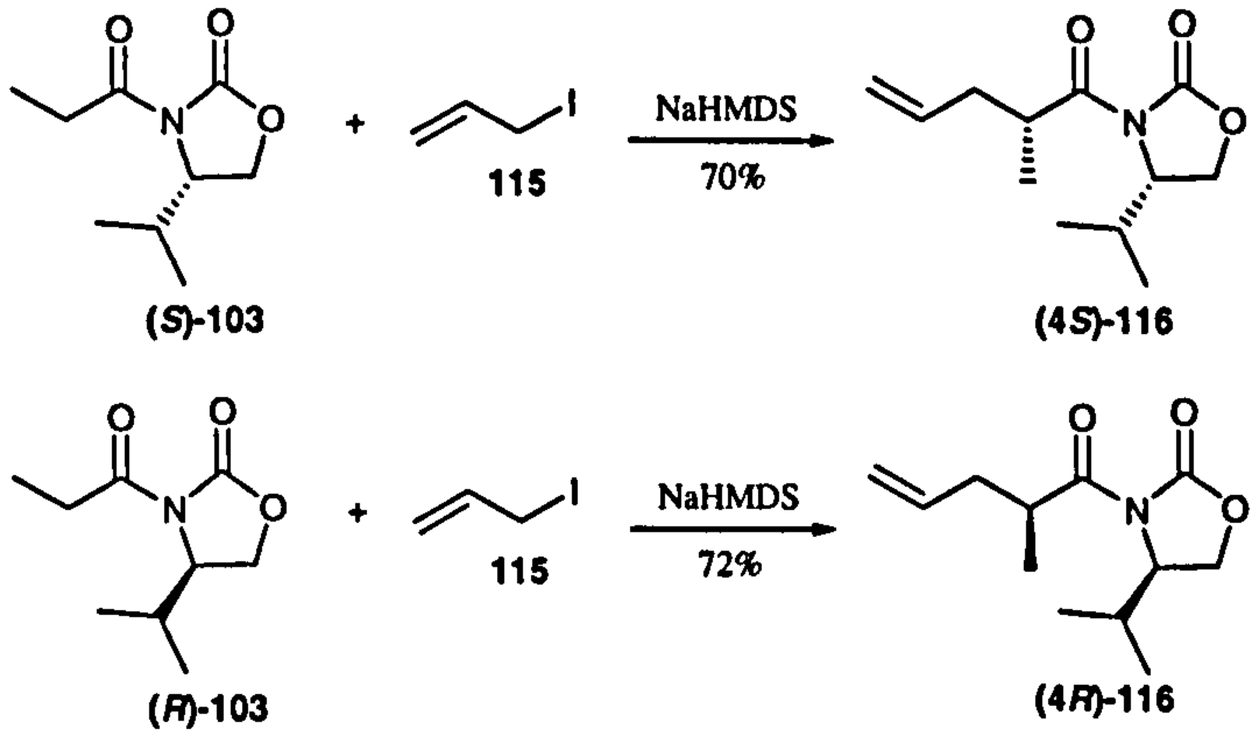


Scheme 46: Attempted alkylation of oxazolidinone **(*S*)-103** with iodide **102**.

	Reagents	Temperature
1.	NaHMDS	-78 °C → R.T.
2.	LiHMDS	-78 °C → R.T.
3.	LDA, HMPA	-78 °C → R.T.
4.	$TiCl_4$, iPr_2NEt	0 °C

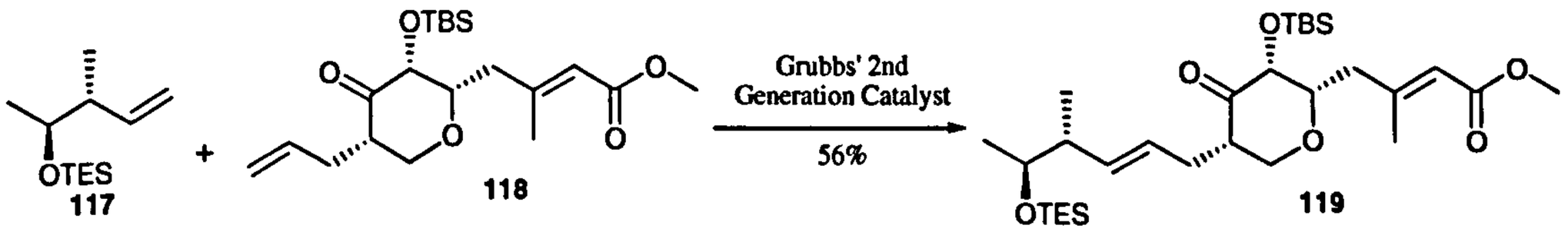
Table 2: Conditions for the alkylation of oxazolidinone **(*S*)-103** with iodide **102**.

These results were disappointing as iodide **102** was successfully employed as an alkylating reagent by McKay[61,88] and alkylation of (*S*)-**103** with allyl iodide **115** has been shown both in the literature[90] and within our own group[91] to proceed in reasonably high yield. Indeed, treatment of (*S*)-**103** with allyl iodide **115** and NaHMDS furnished the desired alkene (*S*)-**116** in 70% yield (Scheme 47). Similarly, allylation of (*R*)-**103** proceeded in 72% yield.

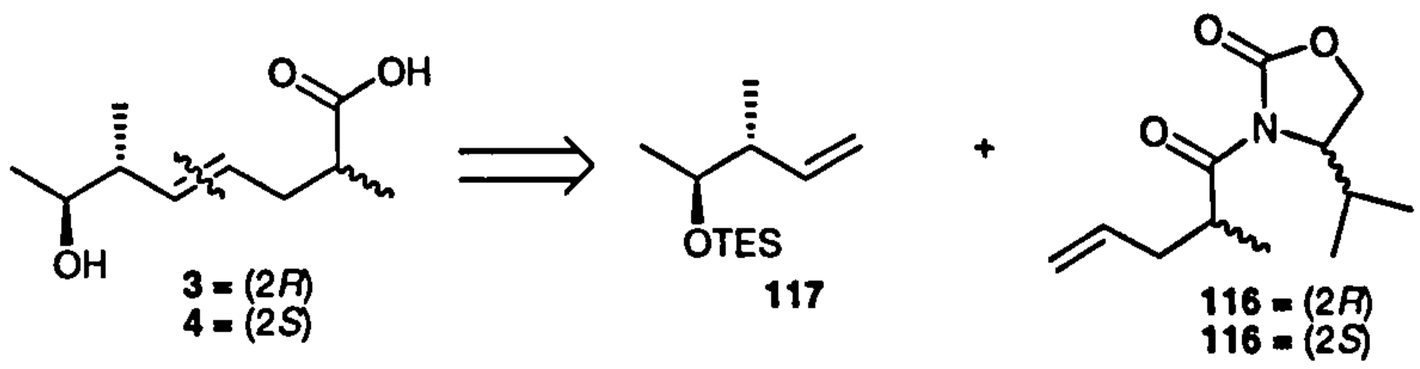


Scheme 47: Alkylation of oxazolidinones (*S*)-**103** and (*R*)-**103** with allyl iodide **115**.

With these compounds in hand, we revised our synthetic approach to the targets **3** and **4**. A recent publication by Marko and co-workers,[62] described installation of the side chain of methyl pseudomonate C by cross metathesis of alkenes **117** and **118**, giving the desired alkene **119** in 56% yield (Scheme 48). Only the *trans* isomer was observed. Applying this to the synthesis of acids **3** and **4**, disconnection at the olefin leads us to alkene **116** and silyl ether **117**, the same alkene used by Marko and co-workers (Scheme 49).

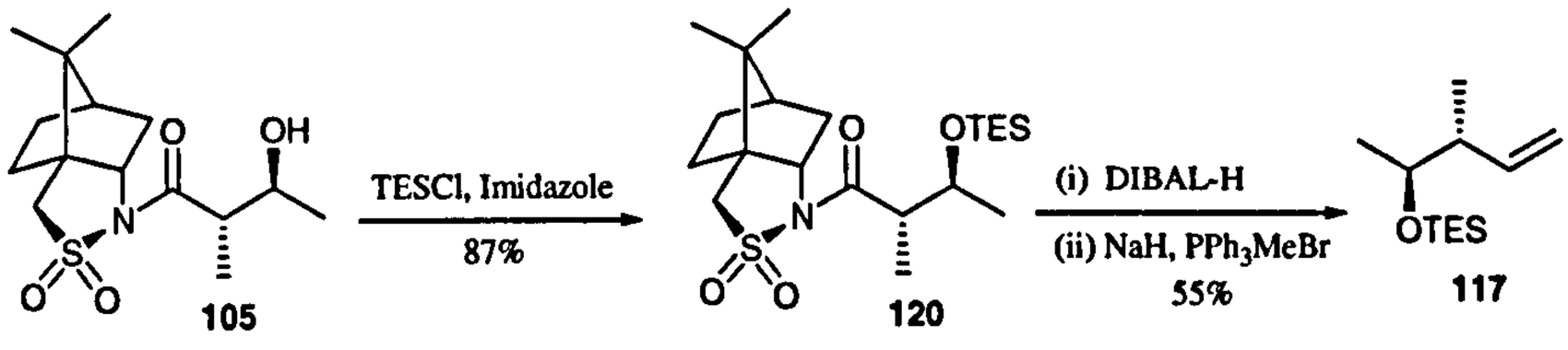


Scheme 48: Marko and co-workers' cross metathesis approach to methyl pseudomonate C.[62]



Scheme 49: Revised retrosynthesis of acids **3** and **4**.

Preparation of alkene **117** was achieved by silyl protection of aldol adduct **105** using chlorotriethylsilane and imidazole to give **120**, followed by cleavage of the auxiliary and a salt free Wittig reaction (Scheme 50). However, some of the labile triethylsilyl group was cleaved during the DIBAL-H reduction leading to a lower yield for this step than might be expected.



Scheme 50: Preparation of alkene **117**.

Cross metathesis of **117** and **(4*S*)-116** proceeded in 50% yield giving a 1:4 mixture of the *cis* **121** and *trans* **123** isomers (Scheme 51). These were found to be inseparable by silica gel column chromatography (even when silver nitrate impregnated silica was employed[92]) or by preparative HPLC, although the latter did lead to partial deprotection of the silyl protecting group. Attempts to isomerise the double bond using iodine[93] or bis-acetonitrile palladium (II) chloride[94] led to a complex mixture of products. It was decided to continue the synthesis with the mixture of isomers in the hope that the final acid would be crystalline and thus purification could be achieved by recrystallisation. Thus, the mixture of **121** and **123** was treated with lithium hydroxide and hydrogen peroxide in order to cleave the auxiliary, followed by acetic acid in THF and water to remove the protecting group furnishing a 4:1 mixture of the desired acid **3** and its *cis* isomer **125** in 40% yield. Again, the isomers were inseparable by column chromatography. Acid **4** was prepared in an analogous manner from alkenes **117** and **(4*R*)-116**, also as a 1:4 mixture of *cis* and *trans* isomers.

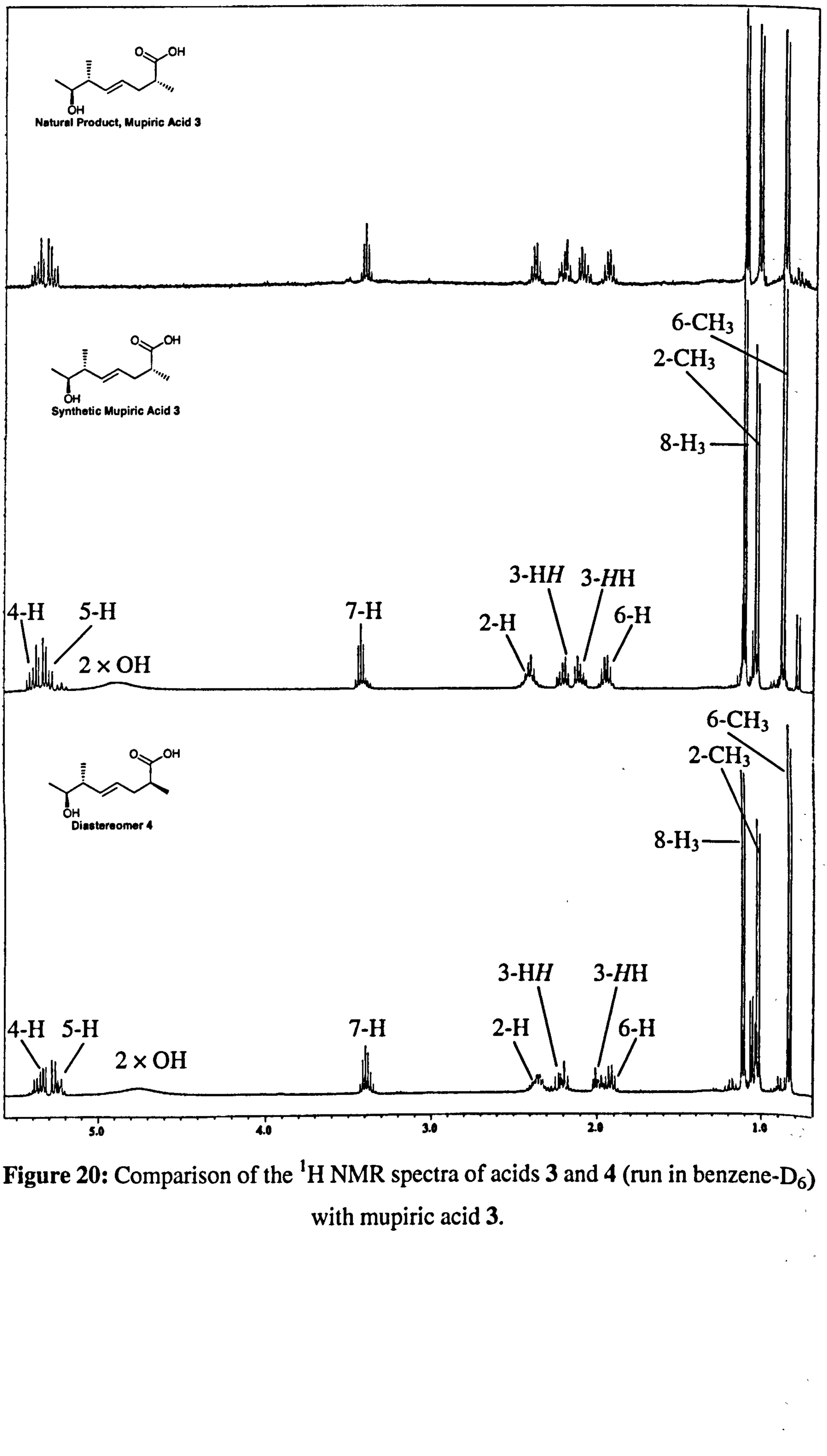
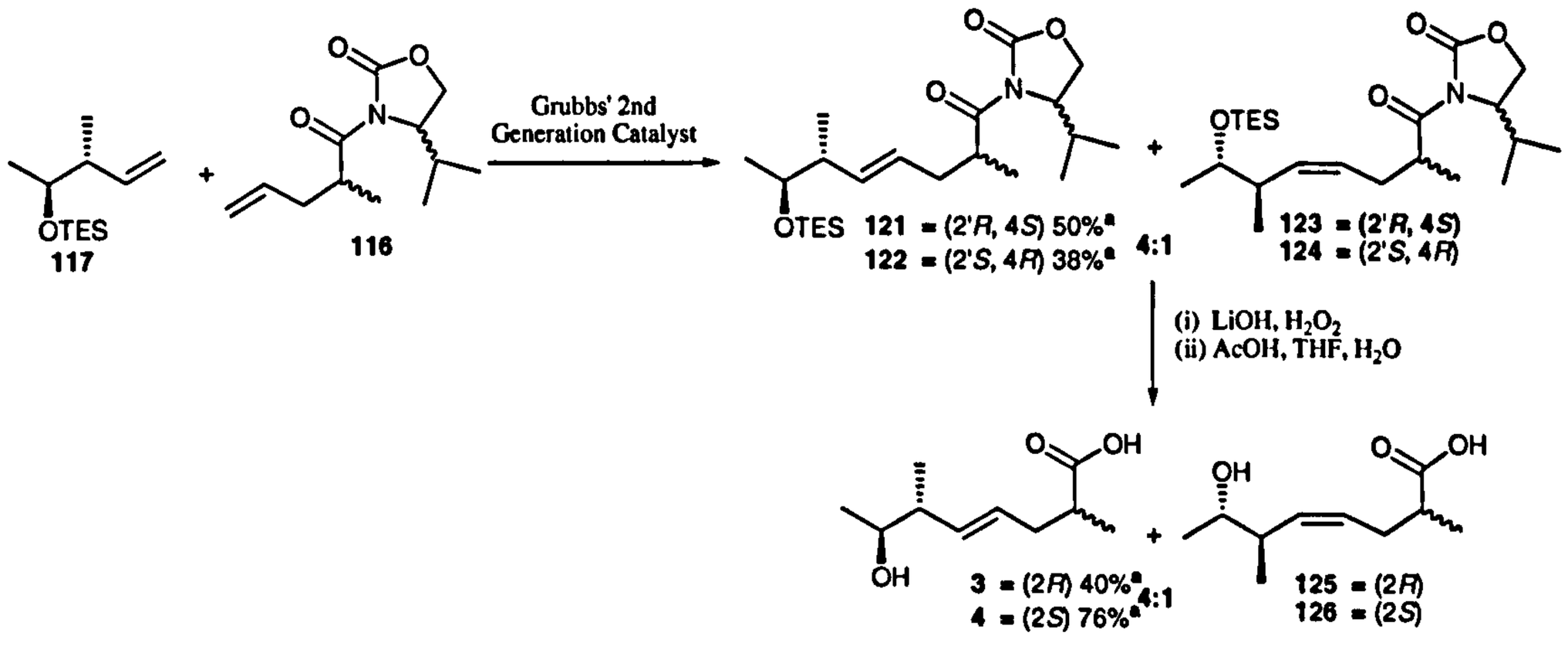


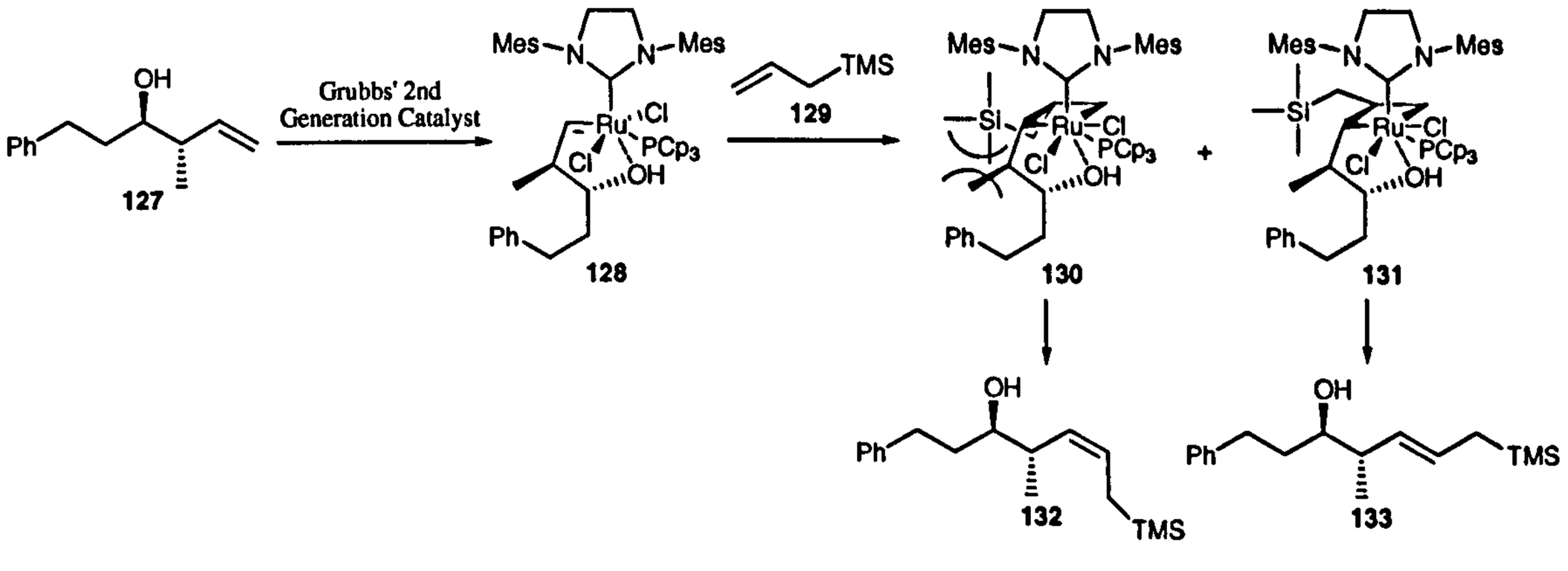
Figure 20: Comparison of the ^{1}H NMR spectra of acids **3** and **4** (run in benzene-D_6) with mupiric acid **3**.



Scheme 51: Cross metathesis of alkenes reaction. [a]Yields quoted are for 4:1 mixtures.

Comparison of the ^{1}H NMR spectra of acids **3** and **4** (run in $CDCl_3$) with that of the isolated metabolite, mupiric acid **3**, was inconclusive. The three spectra were almost identical. Although slightly better correlation of the 2-CH_3 was observed for the 2*R* diastereomer **3**, the difference (approximately 0.002 ppm) was so small that no firm conclusion could be drawn. When the spectra of acids **3** and **4** were run in benzene-D_6 however, they were significantly different (Figure 20). The signals corresponding to 3-*H*H (2.11 ppm) and 3-H*H* (2.22 ppm) in the ^{1}H NMR spectrum of mupiric acid **3**, were more resolved in the spectrum of the C-2 epimer **4**, the former moving upfield towards the 6-H signal and the latter moving downfield towards the 2-H signal. This meant that we could confidently and unequivocally assign the C-2 stereocentre of mupiric acid **3** as the *R* configuration.

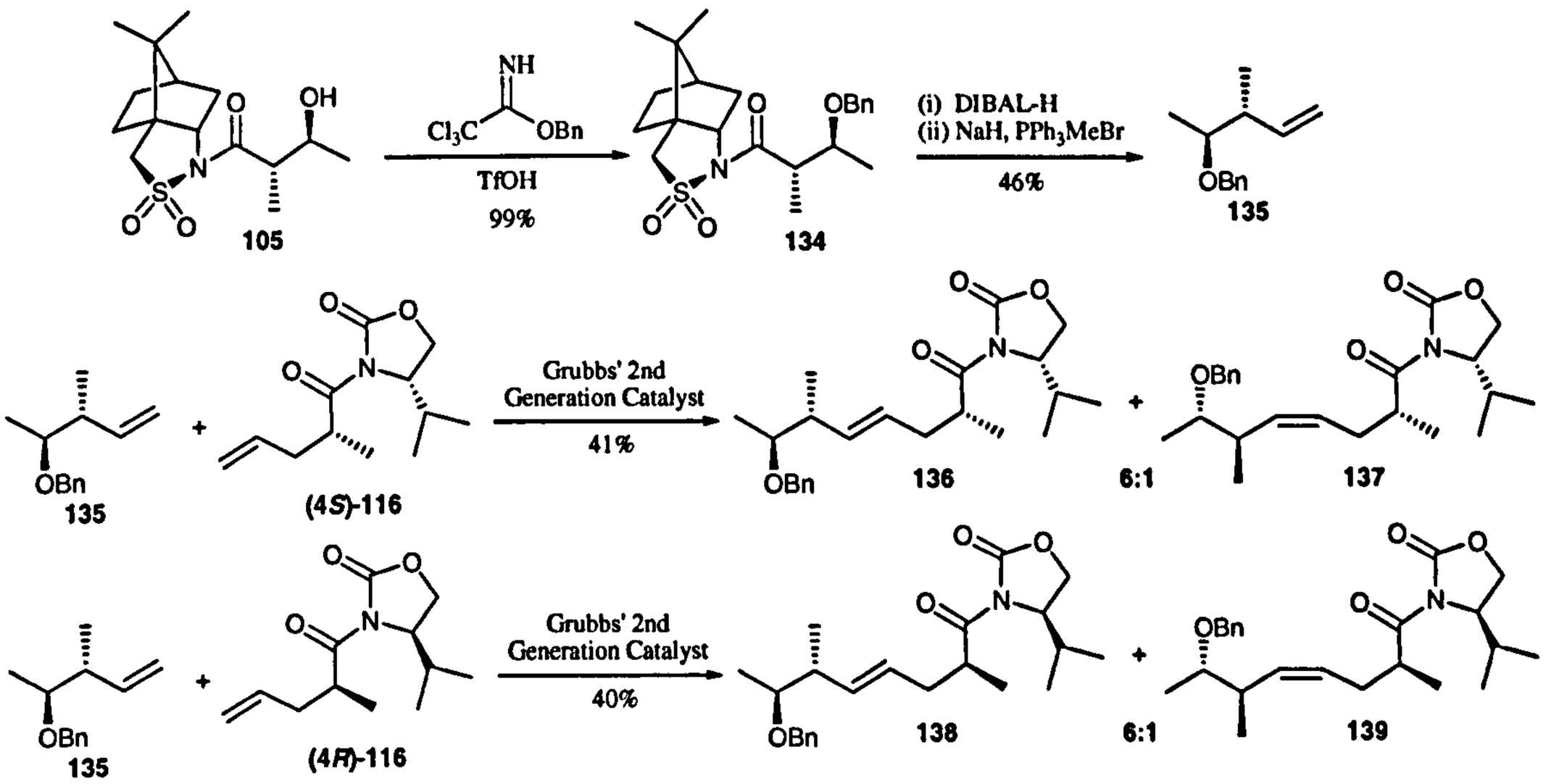
Although the 4:1 mixtures had proved adequate for the structural elucidation of mupiric acid **3**, we were still keen to obtain a pure sample and to develop the methodology for the synthesis of future mutant metabolites. Taylor and co-workers found that increased *trans* selectivity in cross metathesis could be achieved for substrates with a free hydroxyl group *anti* to an alkyl group at the allylic position.[95] Once the substrate **127** has reacted with the catalyst, a lone pair on the oxygen is proposed to chelate to the ruthenium to form a five-membered transition state **128** (Scheme 52). The bulky allyltrimethylsilane **129** approaches from the least hindered α-face leading to two possible intermediates **130** and **131**. The first of these, which leads to the *cis* product **132**, is less favoured due to the steric interaction between the methyl and the trimethylsilyl substituents and thus the *trans* product **133** is favoured.



Scheme 52: Proposed five-membered ring chelate intermediate leading to increased *trans* selectivity.[95]

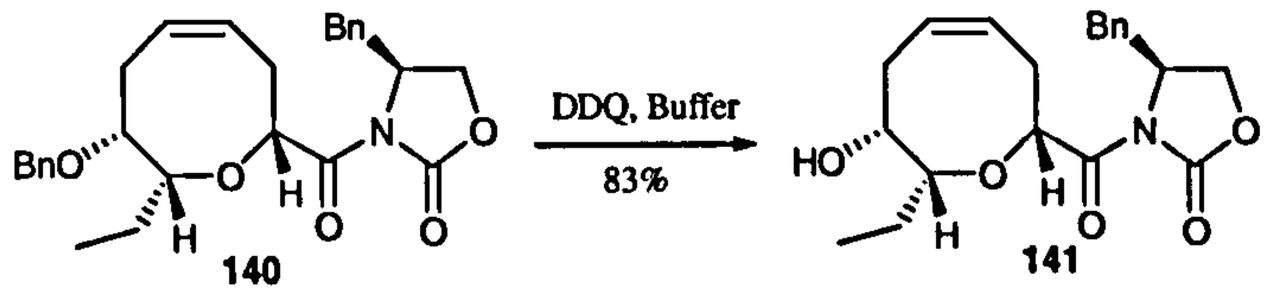
We briefly investigated deprotecting alkene **117** but encountered significant volatility problems and so instead we turned our attention towards the synthesis of the benzyl protected derivative **135**. During some concurrent work (which will be discussed further in section 2.7) we found that benzyl ethers retain a degree of nucleophilicity about the oxygen lone pair. We hoped that the lone pair of the benzyl protected alkene **135** might chelate in a similar manner to the free alcohols reported by Taylor and co-workers.

The benzyl protected alkene **135** was prepared analogously to its silyl counterpart (Scheme 53). Following Widmer's method,[96] alcohol **105** was treated with benzyl 2,2,2-trichloroacetimidate and triflic acid to give benzyl ether **134**. Treatment with DIBAL-H to cleave the auxiliary followed by a salt free Wittig reaction furnished alkene **135** in 46% yield. This then underwent cross metathesis with **(4*S*)-116** to afford a 6:1 *trans/cis* mixture of the benzyl protected oxazolidinones **136** and **137** and with **(4*R*)-116** to afford a 6:1 *trans/cis* mixture of the benzyl protected oxazolidinones **138** and **139**. Both pairs of isomers were inseparable by column chromatography.



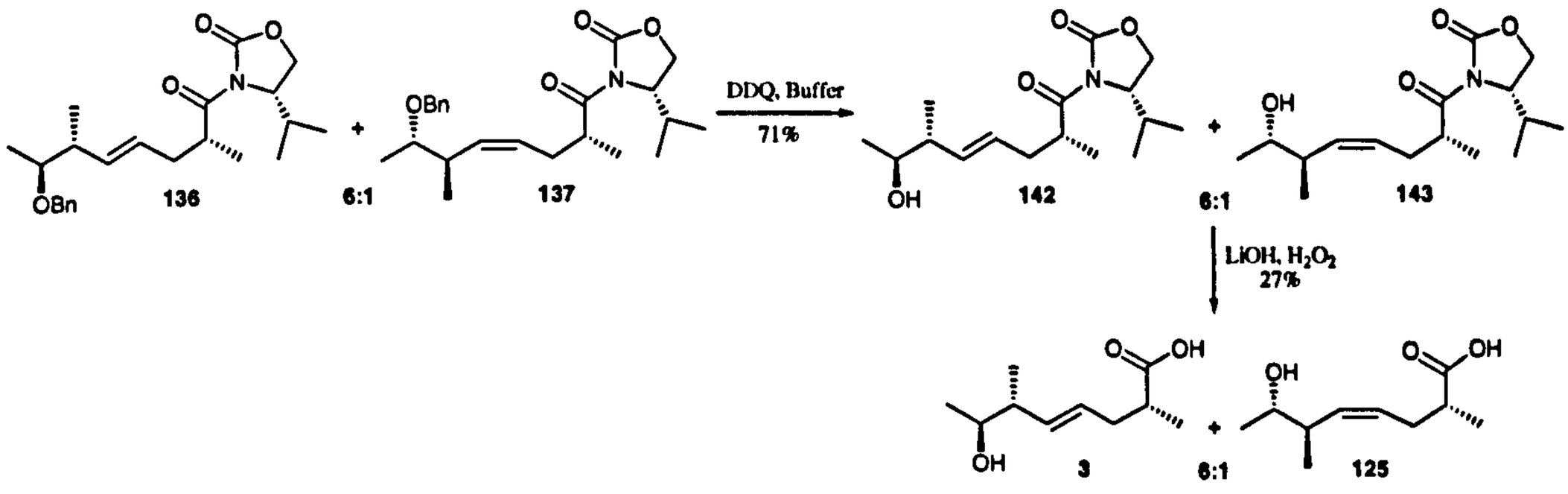
Scheme 53: Cross metathesis of alkene **135** with alkenes **(4*S*)-116** and **(4*R*)-116.**

Although the improvement to the *trans/cis* ratio was not quite as marked as had been hoped, these substrates do have the advantage of a more robust protecting group and so may find applications as intermediates to more complex mupirocin targets. However, in order for them to be useful, it must be possible to deprotect the benzyl ether in the presence of the olefin. In 1999, Crimmins and Emitte reported the use of DDQ (more commonly used for the deprotection of *p*-methoxy- and other substituted benzyl ethers[97]) to deprotect a benzyl ether in the presence of an alkene giving **141** in 83% yield (Scheme 54).[98] Not only was this method mild and high yielding but the substrate employed **140** also contained an oxazolidinone and so was very similar to benzyl ethers **136-139.**



Scheme 54: DDQ deprotection of a benzyl ether in the presence of an alkene.[98]

The mixture of alkenes **136** and **137** was treated with DDQ in the presence of buffer solution for 3 days. The reaction mixture was shaken rather than stirred as this proved a more efficient means of mixing the biphasic heterogeneous solution. Alcohols **142** and **143** were obtained in 71% yield and yet again proved inseparable. Finally, cleavage of the auxiliary with lithium hydroxide and hydrogen peroxide afforded mupiric acid **3** and its *cis* isomer **125** as a 6:1 mixture.



Scheme 55: Preparation of mupiric acid **3** from benzyl ether **136.**

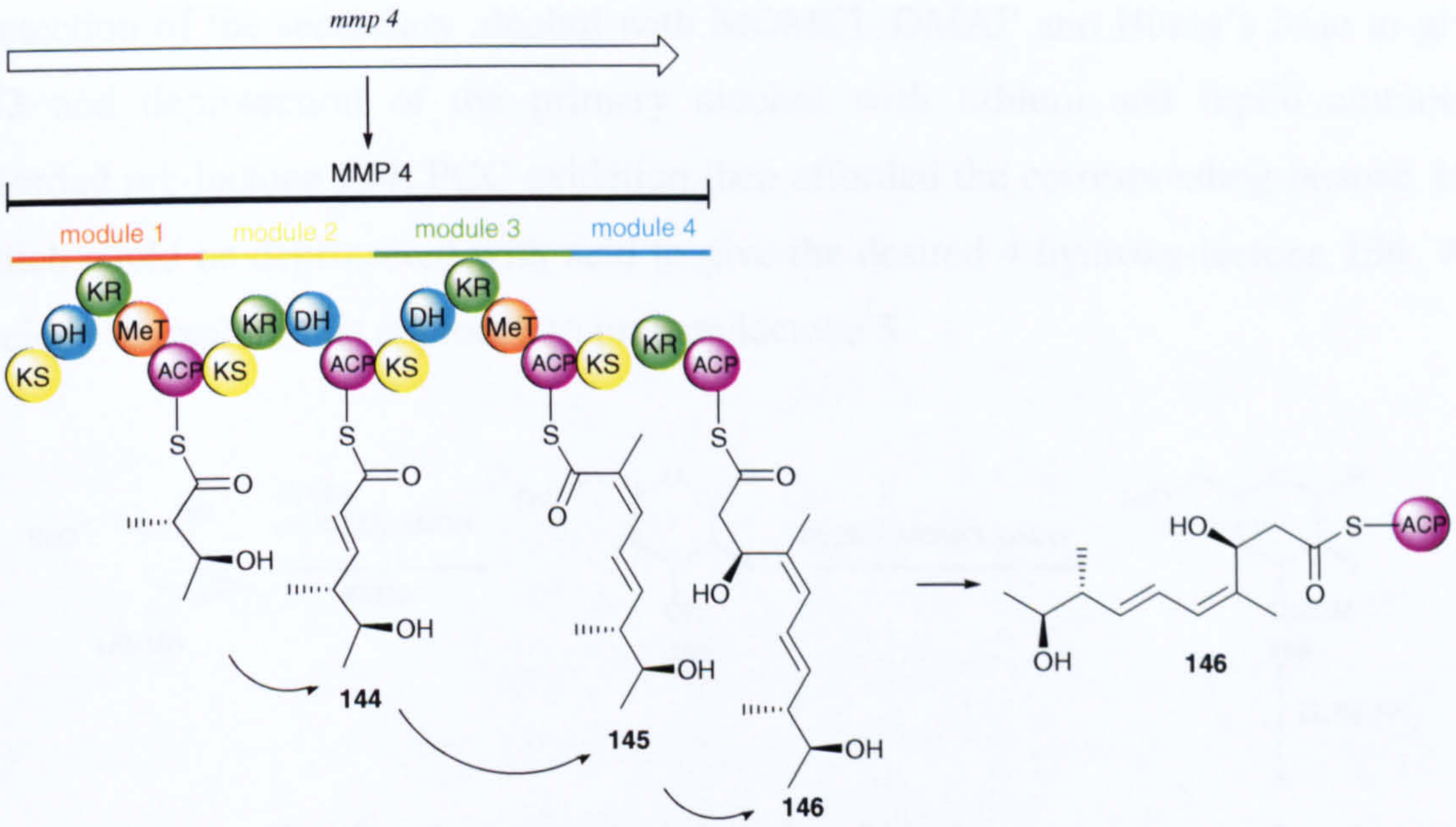
3.3 Studies Towards the Synthesis of the Proposed DH4 Lactone 5

As described in section 1.1, the polyketides follow a logical progression of biosynthesis, allowing putative products, intermediates and mutant metabolites to be predicted. However, the stereochemistry of these compounds is not so easy to predict and often there may be more than one possible stereoisomer. The best way to conclusively prove the structure is to isolate the compound and compare it to authentic material.

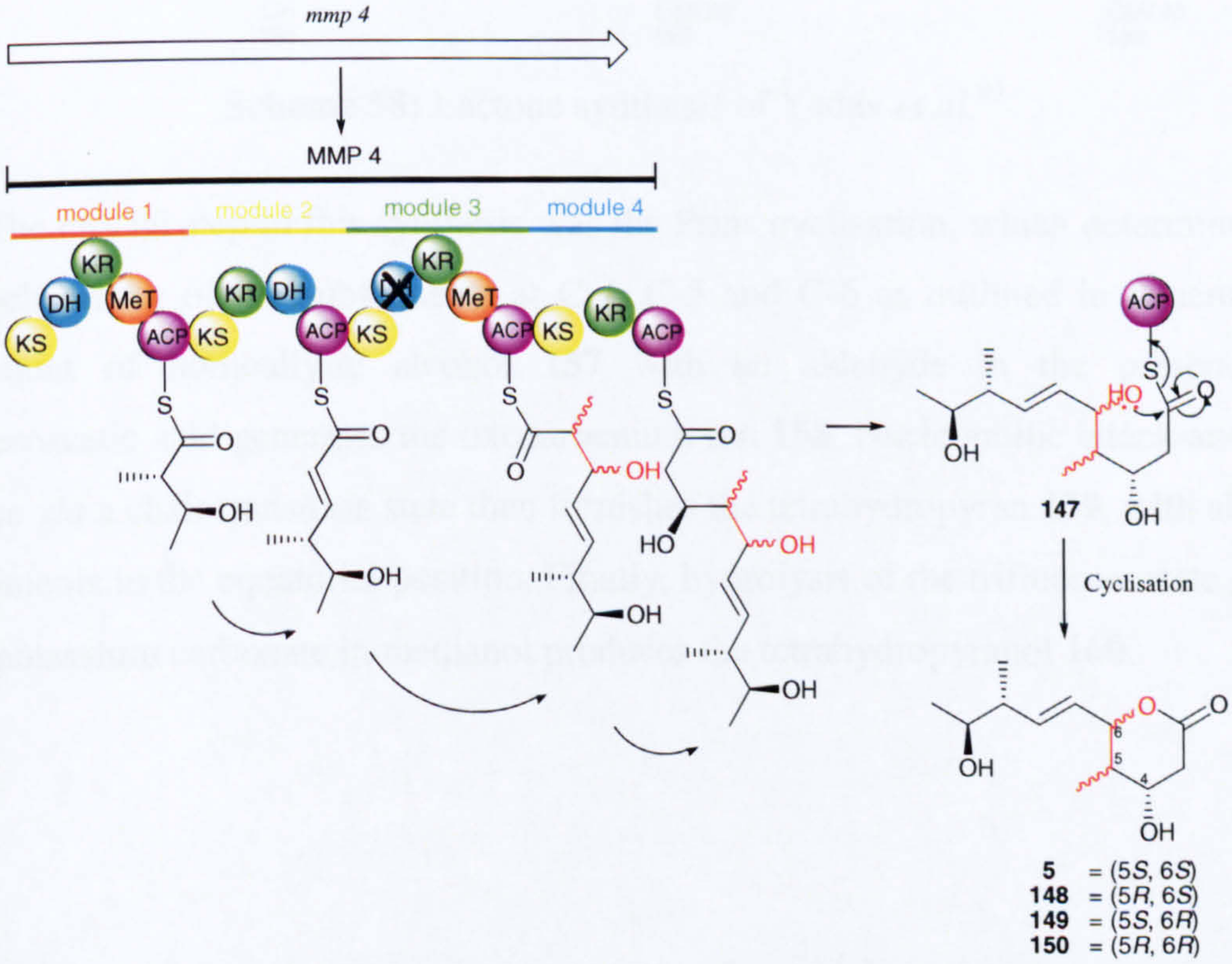
The mupirocin polyketide synthase, discussed in section 3.1 is thought to have many enzyme bound intermediates which are extended with each subsequent condensation cycle and reduced according to the domains present. In module 3 for instance, intermediate **144** is condensed with a malonate extender unit and then the ketone is reduced, dehydrated and methylated to give intermediate **145** (Scheme 56). Intermediate **145** is subsequently extended and the ketone moiety is reduced to give the proposed overall product of MMP4, diene **146**. Genetic mutation of the DH domain in module 3 of the mupirocin PKS, however, is predicted to result in formation of one of four lactones **5, 148, 149** and **150** (Scheme 57). By deleting this domain, reduction of the hydroxyl group in module 3 is unable to take place, thus leaving the carbonyl of the mutant module 4 product **147** open to nucleophilic attack to form a lactone.

In order to assist in the detection and structure elucidation of lactones **5, 148, 149** and **150**, authentic material is required. The stereochemistry of the methyl group at C-5 and the hydroxyl group at C-6 are not known as these are new stereogenic centres and so a flexible synthesis which gives access to all four diastereomers is required. Another reason for our interest in these substrates is that they do not possess a hydroxyl group at the position corresponding to C-6 of the pseudomonic acids (C-3).

As the timing of this oxidation is as yet unknown, we hoped to preempt the discovery of intermediates and mutant metabolites without this hydroxyl group and develop our methodology towards their synthesis. For convenience, we chose to focus initially on lactone **5**.

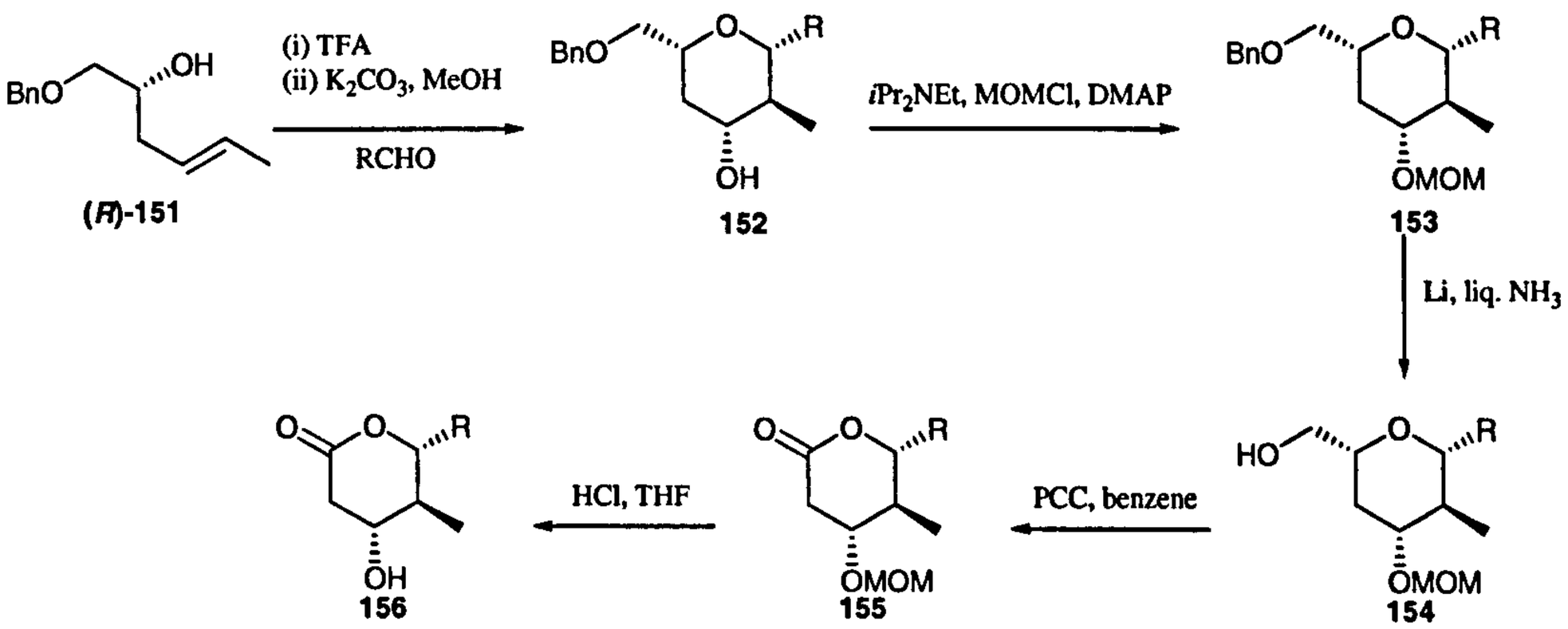


Scheme 56: Predicted formation of intermediate **146** by the mupirocin PKS.



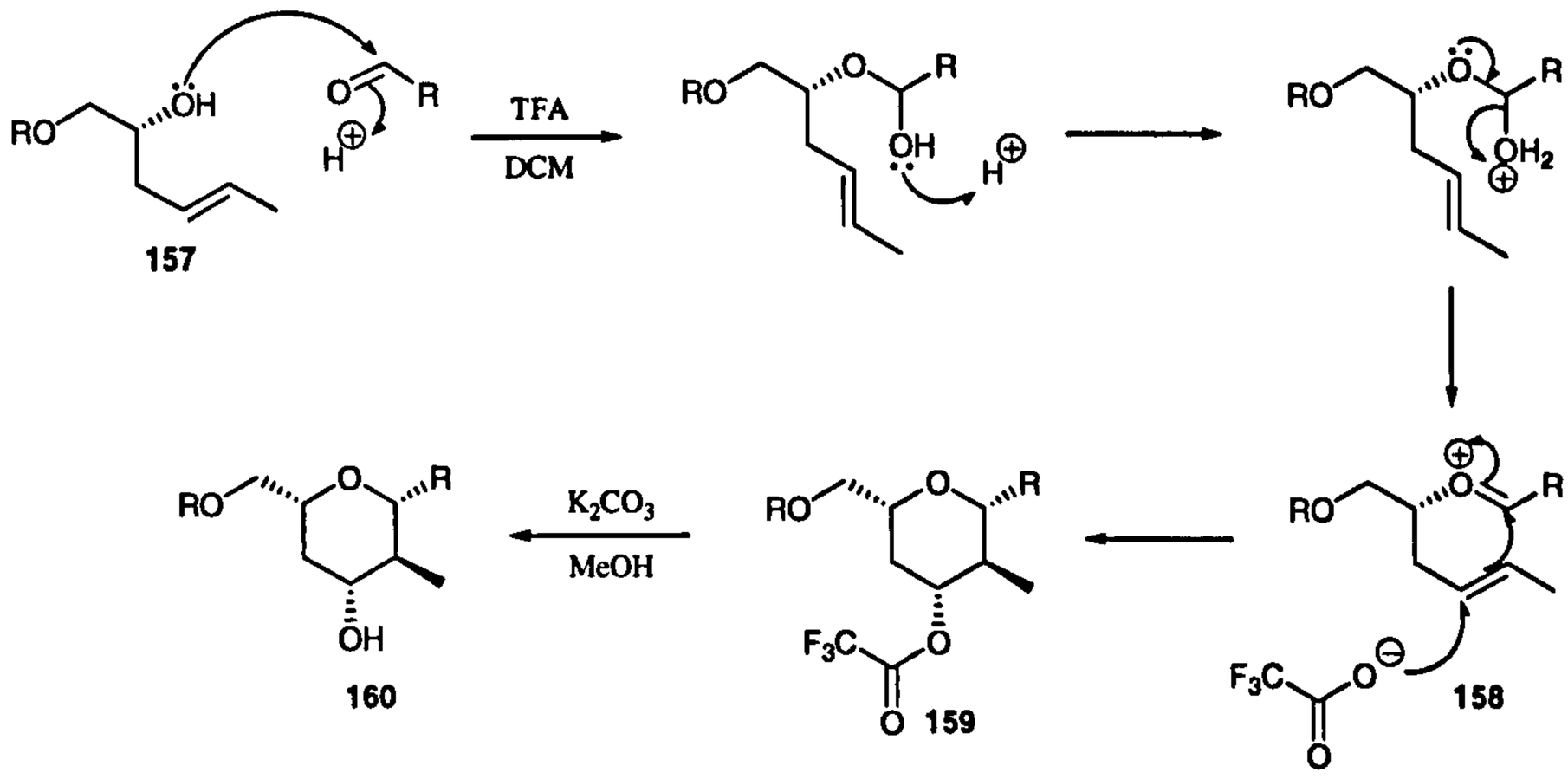
Scheme 57: Predicted lactone formation by the mupirocin PKS following mutation of the DH domain in module 3.

A recent publication by Yadav *et al.*,[99] describes the synthesis of trisubstituted lactones from the corresponding tetrasubstituted tetrahydropyrans **152** (Scheme 58). The latter were accessed *via* a Prins cyclisation of homoallylic alcohol (***R*)-151** and aldehyde precursors, following procedures developed within our group.[100] Subsequent protection of the secondary alcohol with MOMCl, DMAP and Hünig's base to give **153** and deprotection of the primary alcohol with lithium and liquid ammonia afforded pre-lactone **154**. PCC oxidation then afforded the corresponding lactone **155** which could be deprotected with acid to give the desired 4-hydroxy-lactone **156**. We decided to explore this approach to prepare lactone **5**.



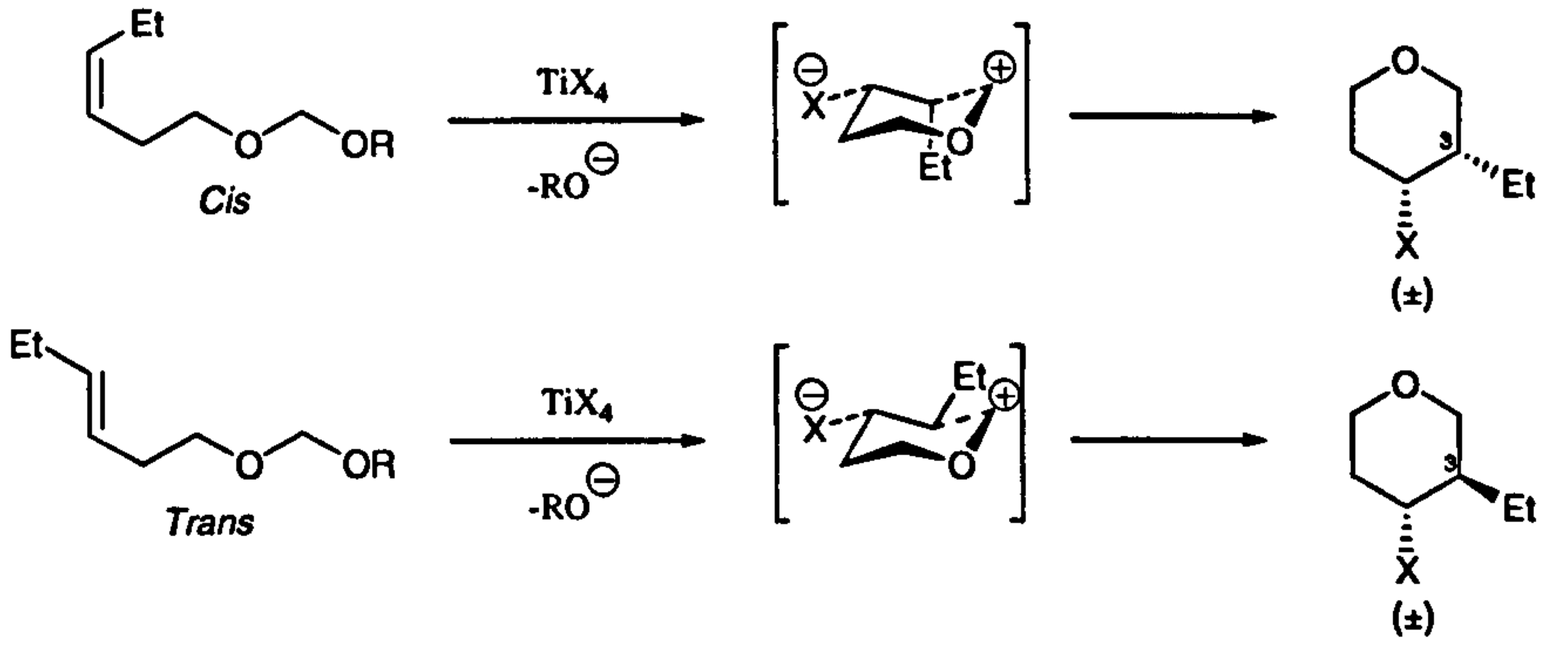
Scheme 58: Lactone synthesis of Yadav *et al.*[99]

The crucial step in this synthesis was the Prins cyclisation, which determines the stereochemistry of the substituents at C-4, C-5 and C-6 as outlined in Scheme 59. Treatment of homoallylic alcohol **157** with an aldehyde in the presence of trifluoroacetic acid generates the oxocarbenium ion **158**. Nucleophilic attack and ring closure *via* a chair transition state then furnishes the tetrahydropyran **159**, with all four substituents in the equatorial position. Finally, hydrolysis of the trifluoroacetate group with potassium carbonate in methanol produces the tetrahydropyranol **160**.



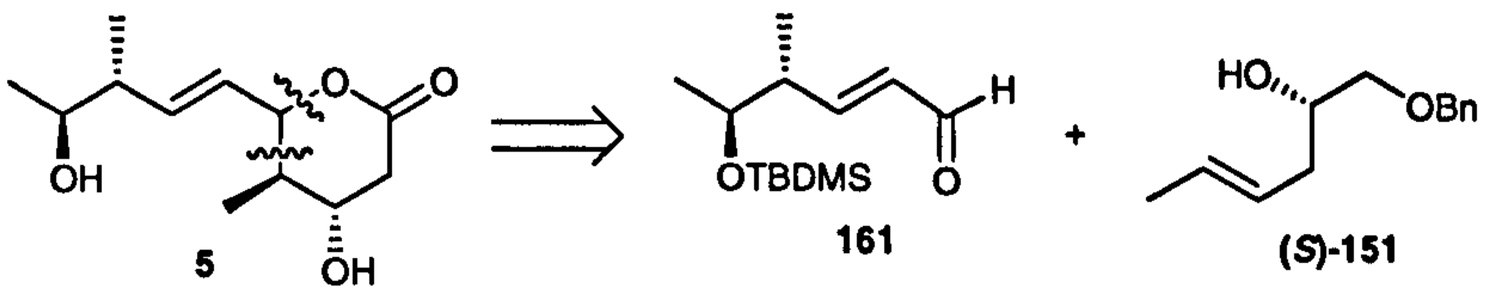
Scheme 59: Prins cyclisation to prepare tetrahydropyrans.

It is possible to invert the centre at C-4 by a Mitsunobu reaction, thus access to both C-4 epimers should be possible. Thompson and co-workers showed that the stereochemistry of the substituent at the C-3 position of the tetrahydropyran ring is determined by the geometry of the homoallylic alcohol employed.[101] Upon generation of the intermediate oxocarbenium ion, the reaction is proposed to proceed *via* a chair-like transition state. It was shown that a *trans* homoallylic alcohol led to an equatorial substituent at C-3, whereas a *cis* homoallylic alcohol led to an axial substituent at C-3 (Scheme 60). We should therefore have access to both of the C-3 epimers simply by varying the geometry of the alkene in the homoallylic alcohol. Thus, access to all four lactones should be possible following the proposed route.



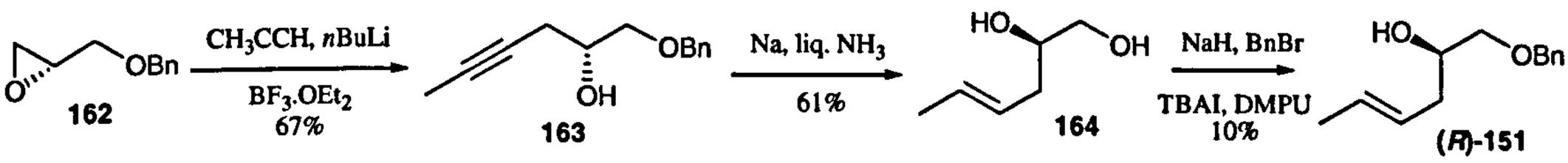
Scheme 60: Stereoselective Prins cyclisation.[101]

Initial retrosynthetic analysis of lactone **5** identified the homoallylic alcohol (*S*)-**151** and aldehyde **161** as suitable precursors (Scheme 61). Both of these segments are known compounds.



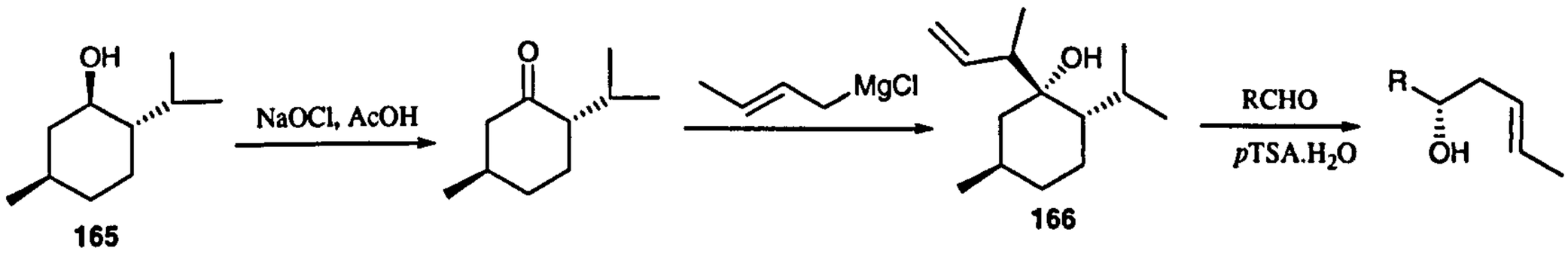
Scheme 61: Initial retrosynthesis of lactone **5**.

Homoallylic alcohol **151** was the substrate employed by Yadav *et al.*[99] in their lactone syntheses. They reported a 64% overall yield and so we investigated the same approach (Scheme 62) working initially with the (*R*)-enantiomer as this was cheaper. Thus, treatment of (2*R*)-benzyl glycidyl ether **162** with propynyllithium in the presence of boron trifluoride diethyl etherate furnished alkyne **163** in 67% yield. Birch reduction of **163** at -78 °C yielded diol **164** in 61% yield. Interestingly, this reaction proceeded with very high stereoselectivity furnishing exclusively the *trans* stereoisomer, as determined by NMR spectroscopy. Crosby,[100] working on an analogous system, obtained an (*E*):(*Z*) ratio of approximately 9:1. Finally, selective protection of the primary hydroxyl group of **164** furnished homoallylic alcohol (***R***)-**151** in 10% yield along with a mixture of di-*O*-benzyl protected material and some starting material (≈76%). The doubly protected material could be converted back to diol **164** and reprotected to increase the overall yield of homoallylic alcohol (***R***)-**151.** However, this method proved tedious and so after several attempts this route was abandoned.



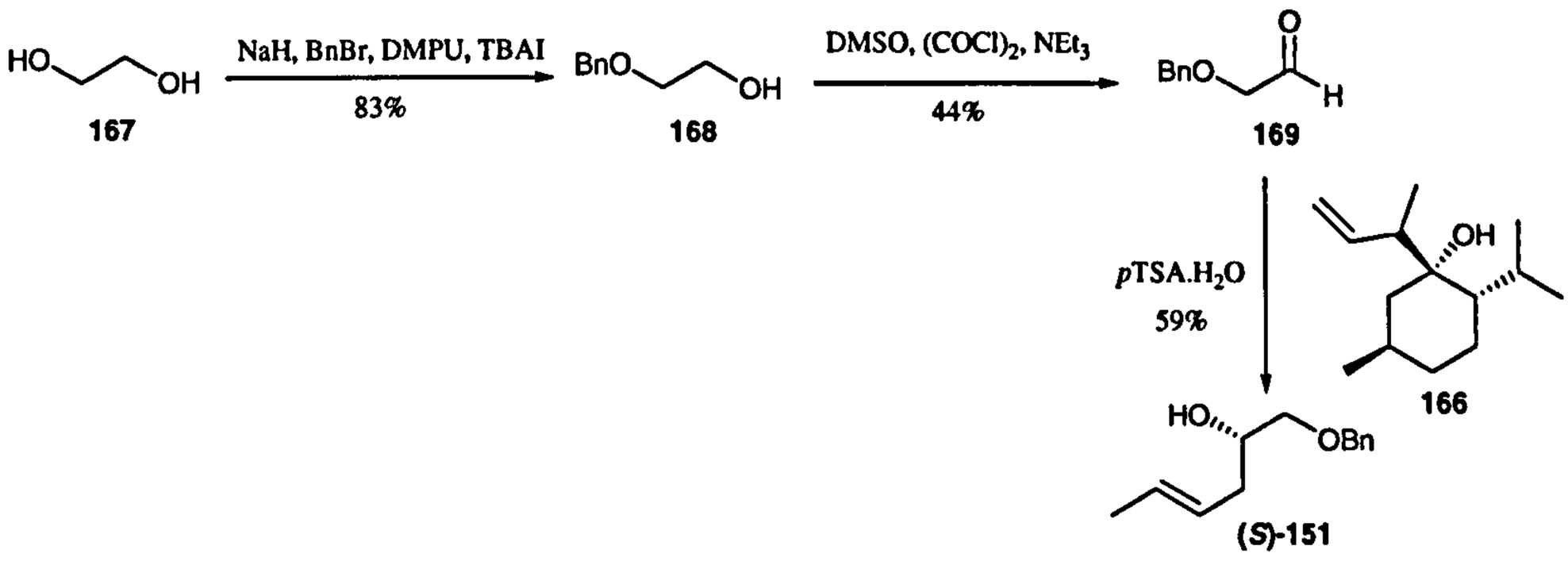
Scheme 62: Synthesis of homoallylic alcohol (***R***)-**151.**

In 2001, Nokami *et al.* reported the asymmetric synthesis of a series of homoallylic alcohols in up to 71% yield and >99% enantiomeric excess, using a chiral crotyl-donor **166** prepared from (-)-menthol **165** (Scheme 63).[102]



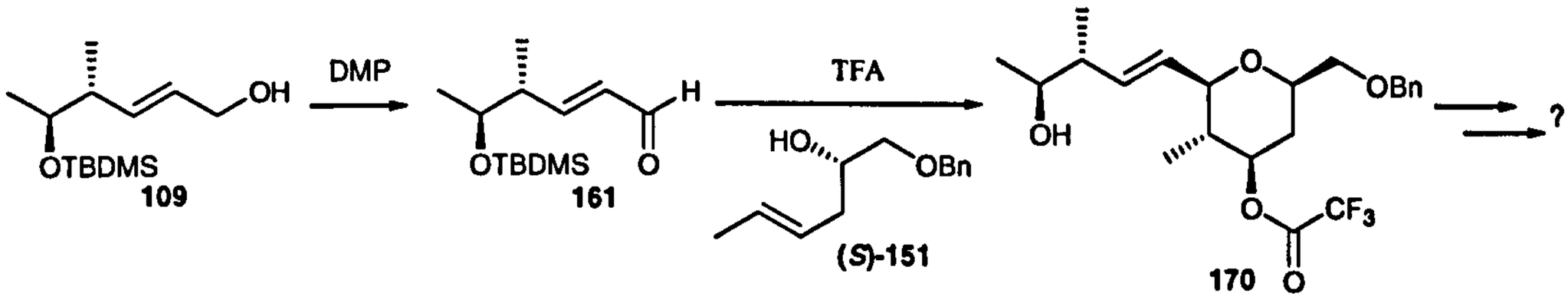
Scheme 63: Crotyl transfer reaction of Nokami *et al.*[102]

As well as being a more efficient route to the desired compound, this route proved a much cheaper alternative, as the chiral component, (-)-menthol **165**, is readily available (as is (+)-menthol). Thus, ethane-1,2-diol **167** was mono-protected using sodium hydride and benzyl bromide to give 2-benzyloxyethanol **168** in 83% yield (Scheme 64). Swern oxidation afforded the aldehyde **169** in 44% yield which underwent crotylation to furnish the desired homoallylic alcohol (*S*)-**151** in 59% yield. Despite numerous attempts, the yields for these last two steps were consistently lower than expected, possibly due to the steric hinderence of the bulky benzyl group.



Scheme 64: Synthesis of homoallylic alcohol (*S*)-**151**.

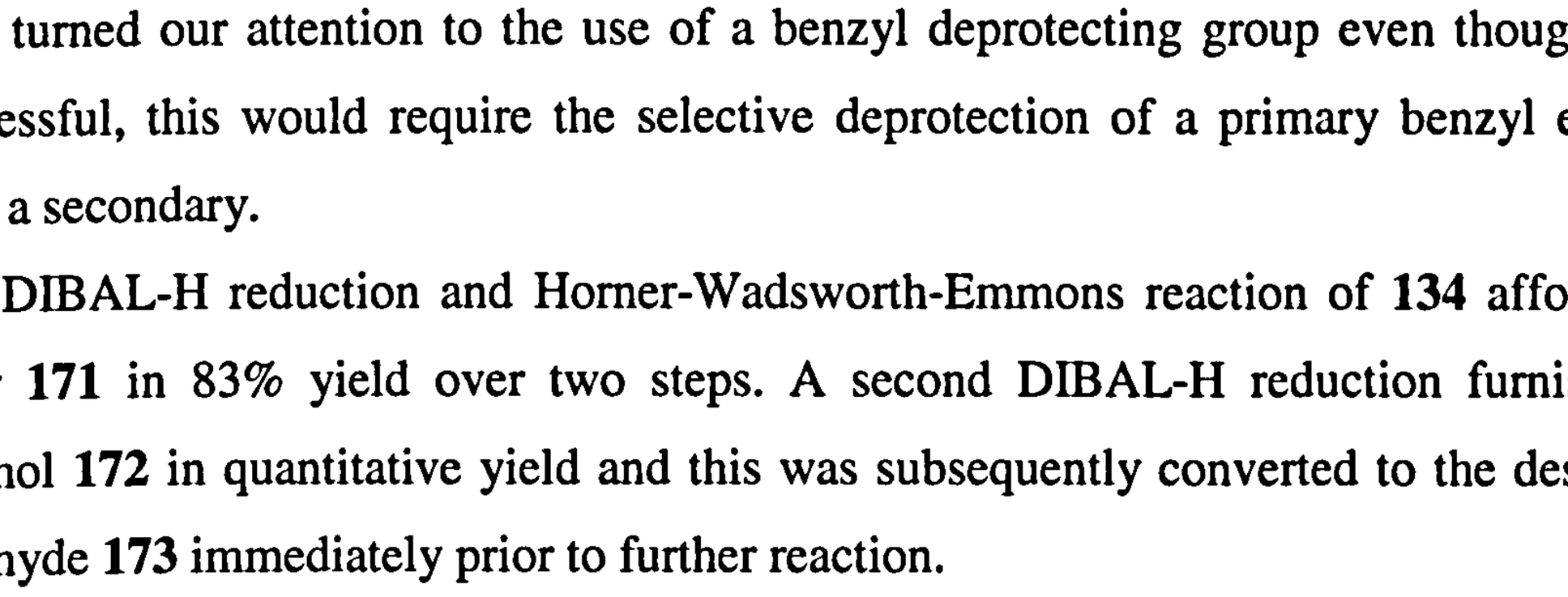
Aldehyde **161** was prepared by a Dess-Martin oxidation of alcohol **109** (Scheme 65). With both precursors in hand, we were ready to investigate Prins cyclisation. A mixture of homoallylic alcohol **151** and aldehyde **161** was treated with TFA and stirred for 4 hours. Although the results of this experiment were difficult to interpret due to the array of products which formed, it was apparent that the silyl protecting group did not stand up to the highly acidic conditions of the reaction. This presented a second homoallylic alcohol which presumably reacted further as no alkene signals were present in the NMR spectra of the products. Some unprotected aldehyde was recovered and (*E*)/(*Z*) isomerisation of the double bond of the starting alcohol (*S*)-**151** was also observed. Whether the desired tetrahydropyran **170** formed at all is speculative as it was not possible to purify the products sufficiently for detailed analysis.



Scheme 65: Treatment of homoallylic alcohol **(*S*)-151** and aldehyde **161** with TFA.

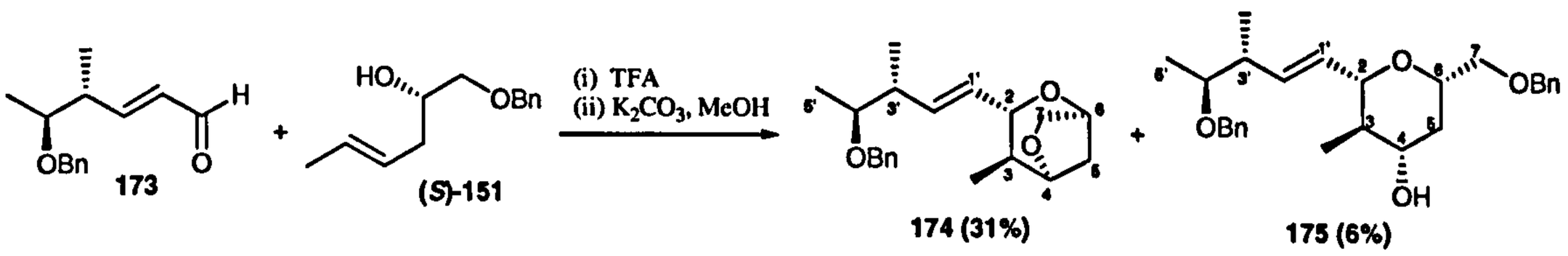
Because of the problem of the TBDMS ether stability in the Prins cyclisation, we next turned our attention to the use of a benzyl deprotecting group even though, if successful, this would require the selective deprotection of a primary benzyl ether over a secondary.

DIBAL-H reduction and Horner-Wadsworth-Emmons reaction of **134** afforded ester **171** in 83% yield over two steps. A second DIBAL-H reduction furnished alcohol **172** in quantitative yield and this was subsequently converted to the desired aldehyde **173** immediately prior to further reaction.



Scheme 66: Preparation of aldehyde **173**.

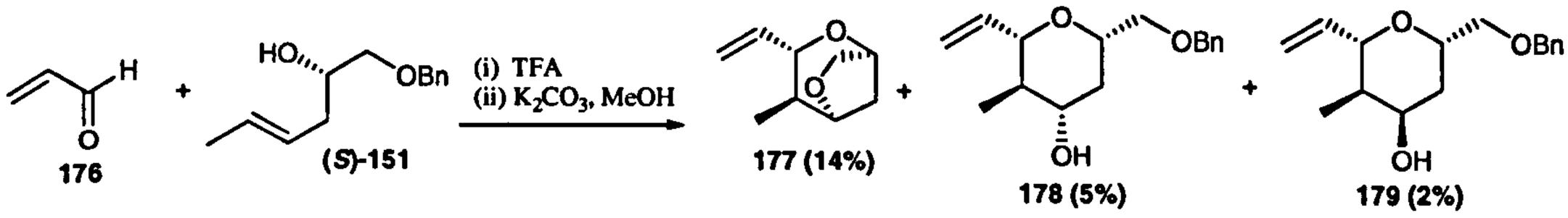
Prins cyclisation of homoallylic alcohol **(*S*)-151** and aldehyde **173** proved very interesting (Scheme 67). Although the desired tetrahydropyran **175** was formed (albeit in a low 6% yield), a more abundant product was isolated in 31% yield. This product did not have an OH stretch in the IR spectrum and had a mass of 316 (rather than the expected mass of 334). The coupling patterns and constants for 3-H, 4-H, 5-H_{ax} and 5-H_{eq} in the ^{1}H NMR spectrum, indicated that the 4-H was being held at an angle that did not permit coupling except to 5-H_{eq}. Furthermore, the aromatic signals in the ^{1}H NMR spectrum integrated to 5 rather than 10 and long range coupling was observed between 4-H and 7-H_2 in the HMBC spectrum. After careful consideration of all the analytical data the major product was assigned as bicycle **174**.



Scheme 67: Prins cyclisation using aldehyde **173** and homoallylic alcohol (*S*)-**151**.

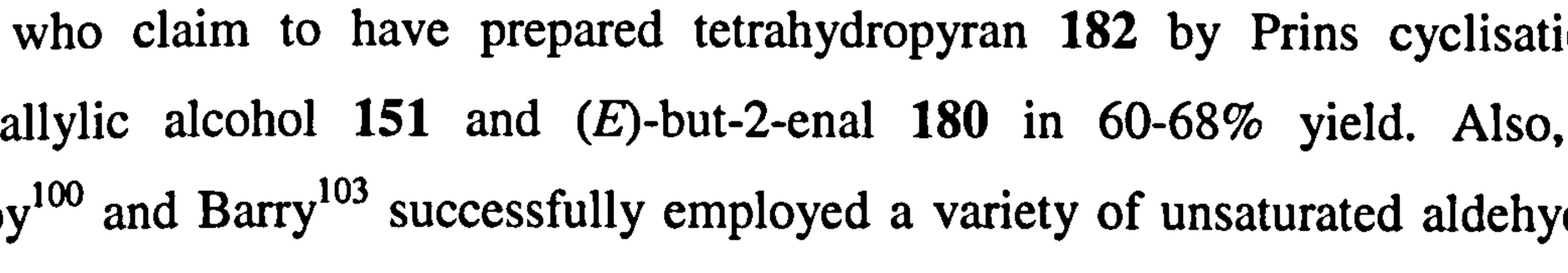
In order to investigate the reaction further, alcohol (*S*)-**151** was treated with a more readily available aldehyde. Propenal was chosen on the grounds that if we could construct the tetrahydropyran core using Prins chemistry, perhaps we could then couple it to the required sidechain by either cross metathesis or a Wittig reaction.

Homoallylic alcohol (*S*)-**151** and propenal **176** in the presence of TFA gave the desired tetrahydropyran **178** in only 5% yield, the C-4 epimer **179** in 2% yield and the bicyclic ether **177** in 14% yield.



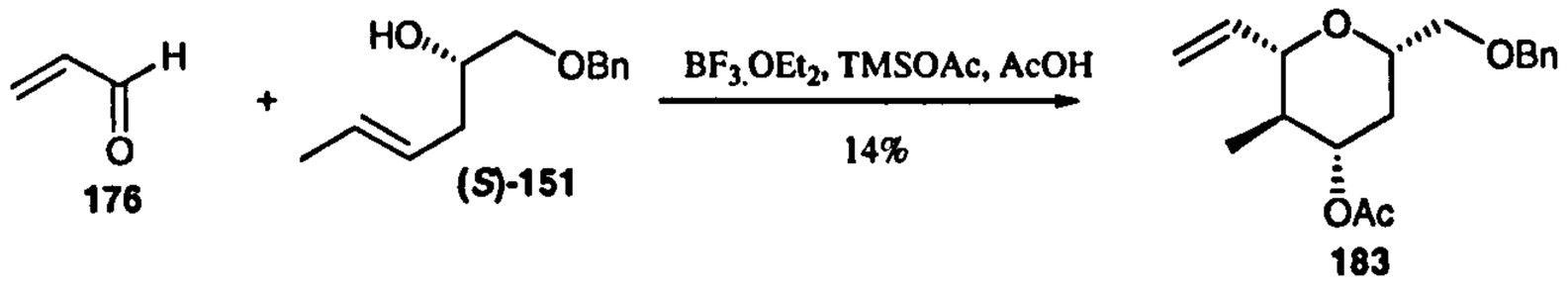
Scheme 68: Prins cyclisation of propenal **176** and homoallylic alcohol (*S*)-**151**.

Both these results seemed to be at odds with the results published by Yadav *et al.*,[99] who claim to have prepared tetrahydropyran **182** by Prins cyclisation of homoallylic alcohol **151** and (*E*)-but-2-enal **180** in 60-68% yield. Also, both Crosby[100] and Barry[103] successfully employed a variety of unsaturated aldehydes in the Prins cyclisation achieving yields of 47-97% and 88% respectively. At this point we decided to repeat Yadav's reaction and although tetrahydropyran **182** did form in 7% yield, the major product was bicycle **181**, which formed in 59% yield (Scheme 69).



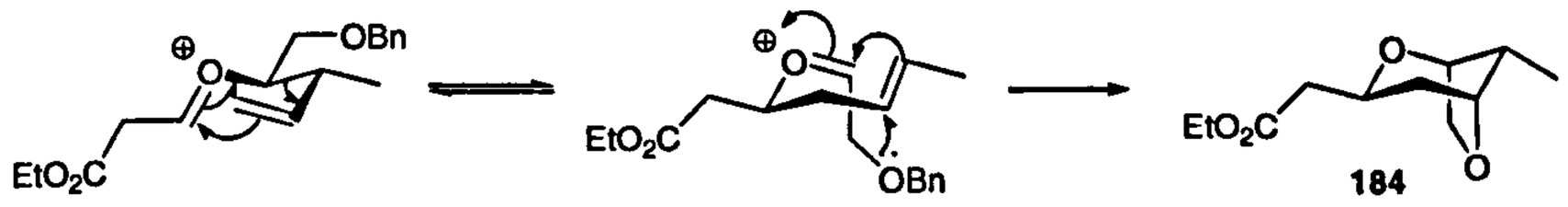
Scheme 69: Preparation of tetrahydropyran **182**.

Changing the Prins conditions to boron trifluoride diethyl etherate, acetic acid and trimethylsilyl acetate (as a fluoride trap) increased the yield of the desired tetrahydropyran **183** to 14% (Scheme 70). However, although no bicycle formation was observed using these conditions, the yield was nevertheless inadequate.



Scheme 70: Prins cyclisation of propenal **176** and homoallylic alcohol (*S*)**-151** using $BF_3.OEt_2$ conditions.

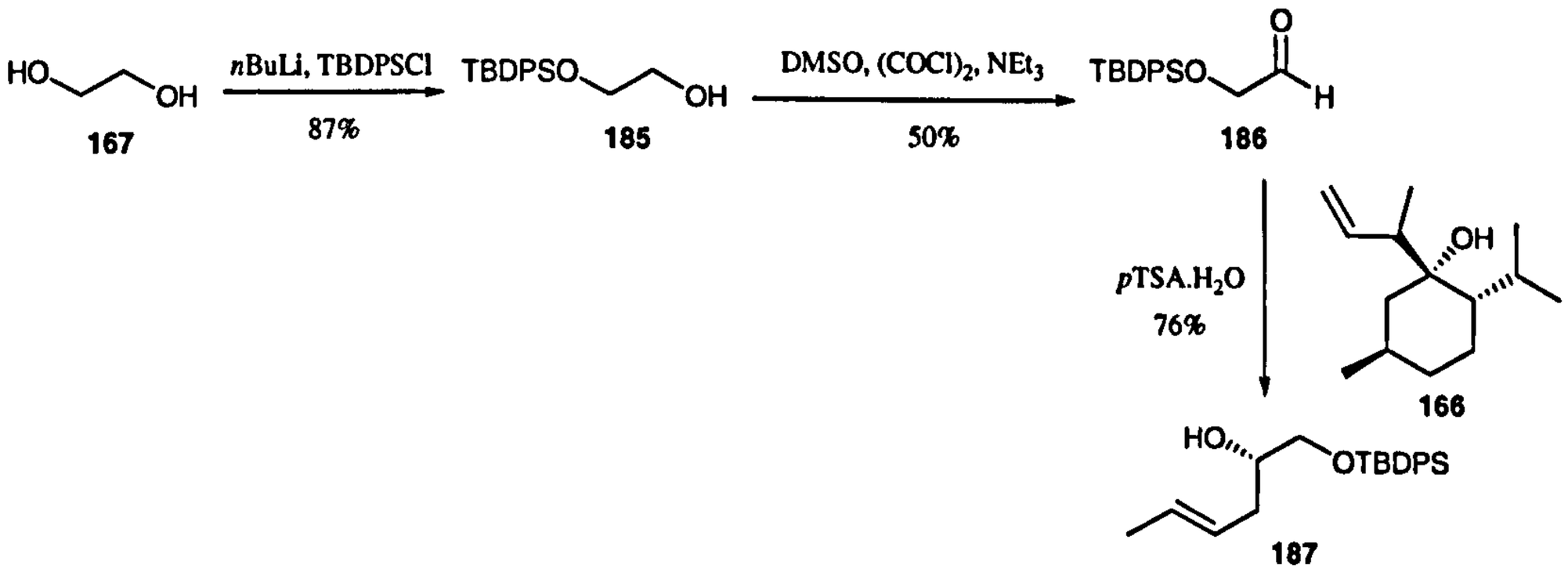
Hart *et al.*[104] reported the synthesis of bicyclic ethers during the synthesis of trisubstituted tetrahydropyrans by Prins cyclisation from enol ethers. They proposed nucleophilic attack of the benzyl oxygen onto the oxycarbenium ion in place of trifluoroacetate as the likely mechanism for the formation of **184** (Scheme 71).



Scheme 71: Formation of bicyclic ether **184** as proposed by Hart *et al.*[104]

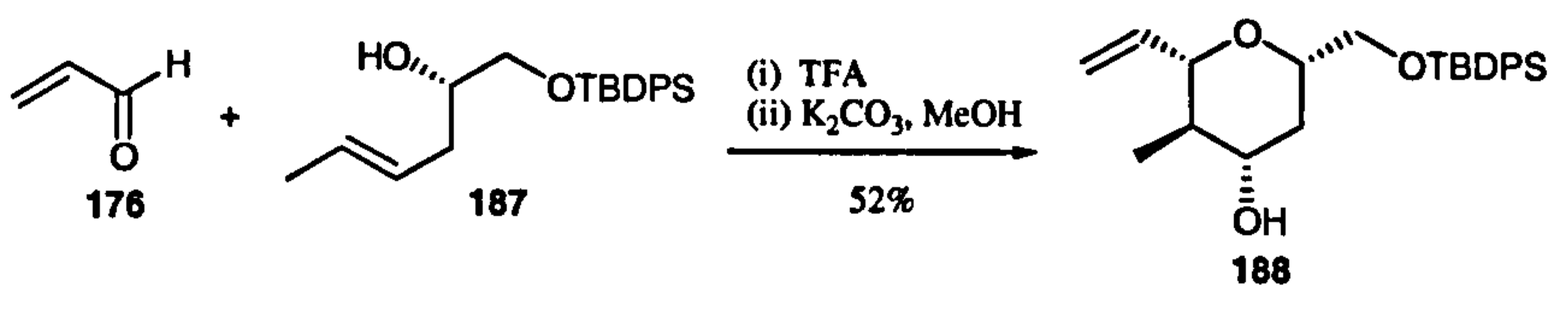
Hart *et al.* found that using a bulkier protecting group in place of the benzyl ether prevented this intramolecular attack from occurring. Their chosen protecting group was *tert*-butyldiphenylsilyl ether and so we decided to follow suit.

Homoallylic alcohol **187** was prepared as shown in Scheme 72. Ethane-1, 2-diol **167** was mono-protected as the silyl ether **185**. Swern oxidation of **185** afforded aldehyde **186** which underwent alkylation to furnish the desired homoallylic alcohol **187** in 76% yield. Initial yields for this crotylation step were in the region of 16%. However increasing the reaction time to 3 days dramatically increased the yield.



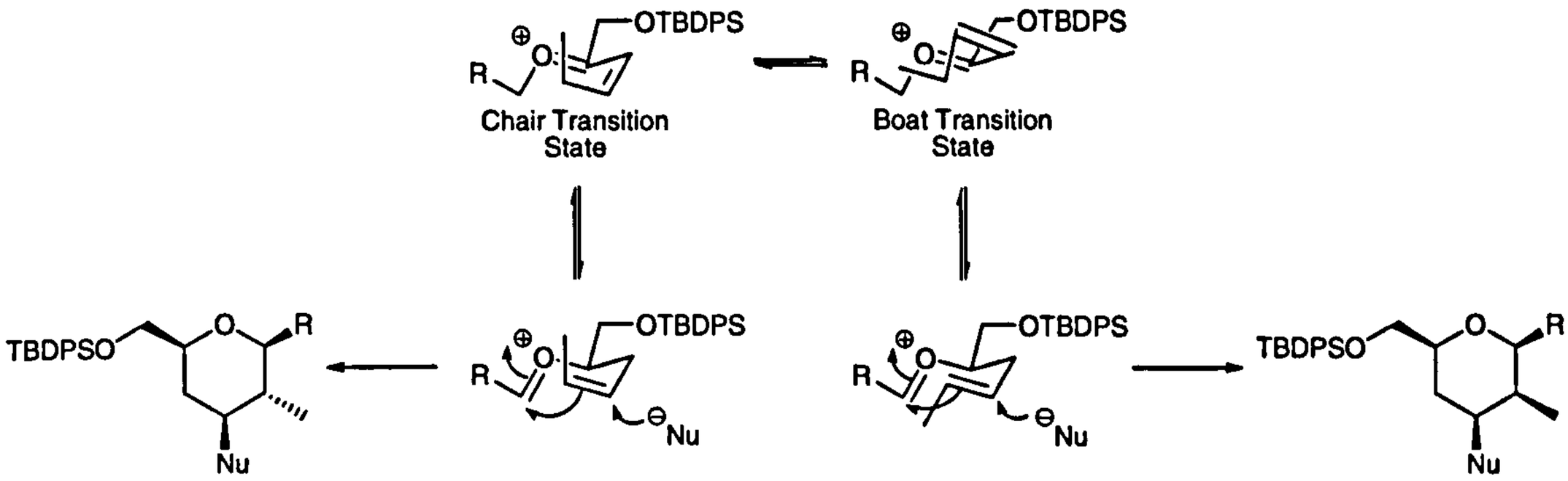
Scheme 72: Preparation of homoallylic alcohol **187.**

Treatment of homoallylic alcohol **187** with propenal **176** and TFA gave, after hydrolysis, an array of unidentifiable products, from which the desired tetrahydropyran **188** was isolated in 11% yield. On a positive note, no bicycle was produced using the new protecting group. Increasing the quantity of aldehyde from 4 to 10 equivalents, resulted in a 52% yield of the desired compound (slightly contaminated with its C-3 epimer) and no side-products were observed. However, the reaction did not go to completion and (*E*)/(*Z*)-isomerisation of the starting material was again observed.



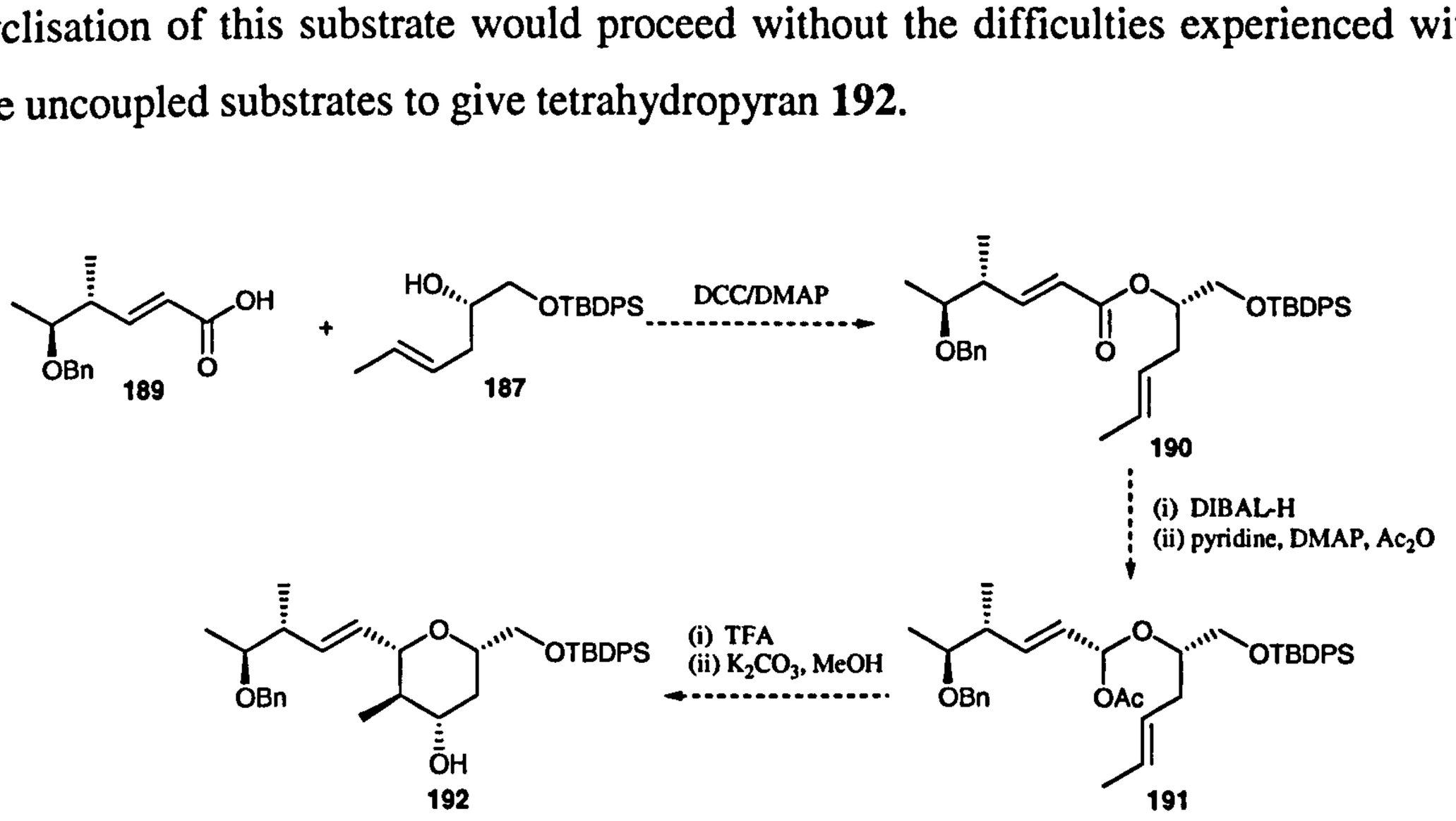
Scheme 73: Prins cyclisation using propenal **176** and homoallylic alcohol **187.**

The results from all these experiments seem to suggest that the Prins cyclisation towards the desired compound is not occurring fast enough, leading to isomerisation, side reactions and rearrangements. Rychnovsky *et al.* noted the formation of C-3 epimers during Prins cyclisation and proposed a 2-oxonia-Cope rearrangement through a boat transition state, as the likely cause (Scheme 74).[105] They investigated ways of increasing the rate of the reaction (as this denies the competing 2-oxonia-Cope rearrangement time to occur) and successfully overcame the problem using α-acetoxy ethers and their segment-coupling approach.[106]



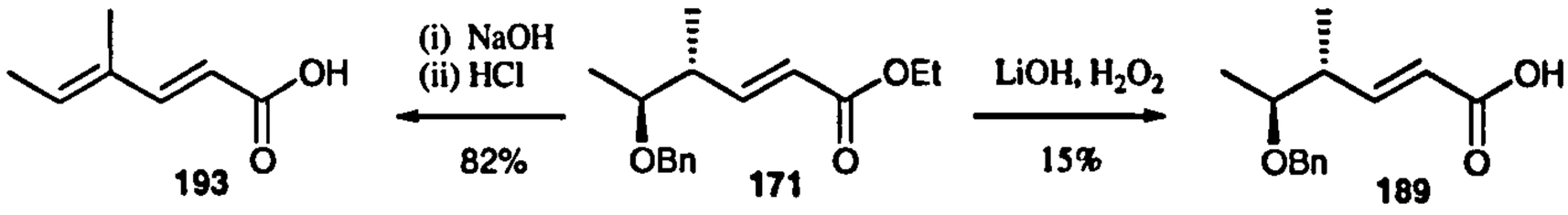
Scheme 74: 2-Oxonia-Cope rearrangement through chair and boat transition states.[105]

Segment-coupling,[107] as the name suggests, involves coupling the two Prins reactants together prior to cyclisation. It was proposed, that esterification of acid **189** and homoallylic alcohol **187** to give ester **190** followed by reductive acetylation would give access to the α-acetoxy ether **191** (Scheme 75). It was hoped that Prins cyclisation of this substrate would proceed without the difficulties experienced with the uncoupled substrates to give tetrahydropyran **192.**



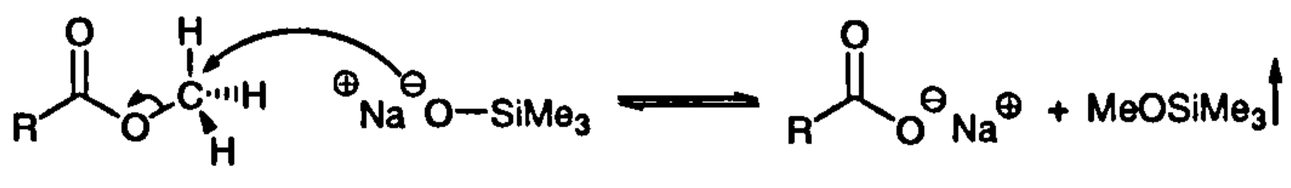
Scheme 75: Proposed segment-coupling approach to Prins cyclisation.

Acid **189** proved a more challenging synthetic target than might be expected. For example, hydrolysis of ester **171** with sodium hydroxide resulted in elimination to give dienoic acid **193** (Scheme 76). Using milder conditions such as lithium hydroxide and hydrogen peroxide however resulted in a sluggish reaction which only gave the target acid **189** in 15% yield, even after a week.



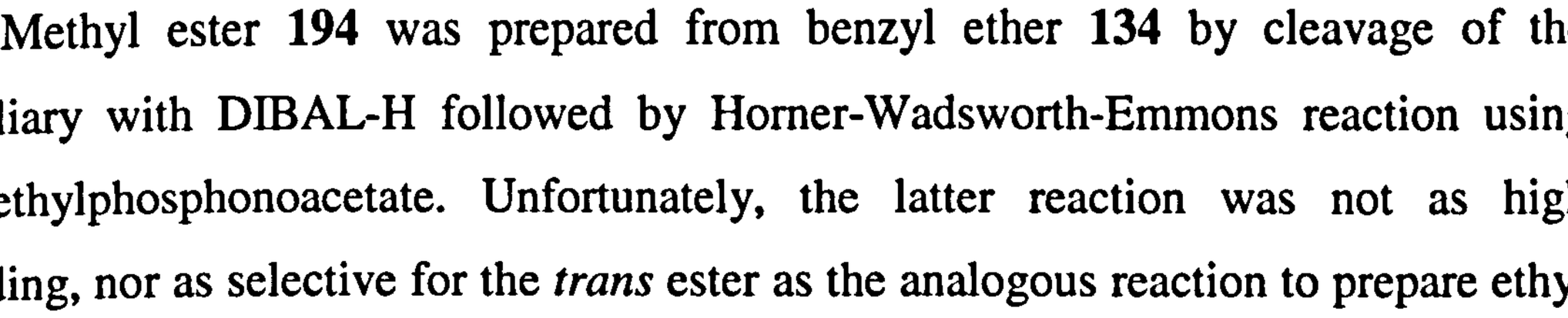
Scheme 76: Hydrolysis of ester **171**.

Laganis and Chenard reported the use of sodium trimethylsilanoate to convert esters and other derivatives to the sodium salts of the corresponding carboxylic acids.[108] Methyl esters, acyl fluorides and trimethylsilyl acetates were all converted to their corresponding sodium salts in excellent yields. In the case of the methyl ester, the reaction is proposed to proceed *via* nucleophilic attack of the methoxy moiety by the silanoate (Scheme 77).



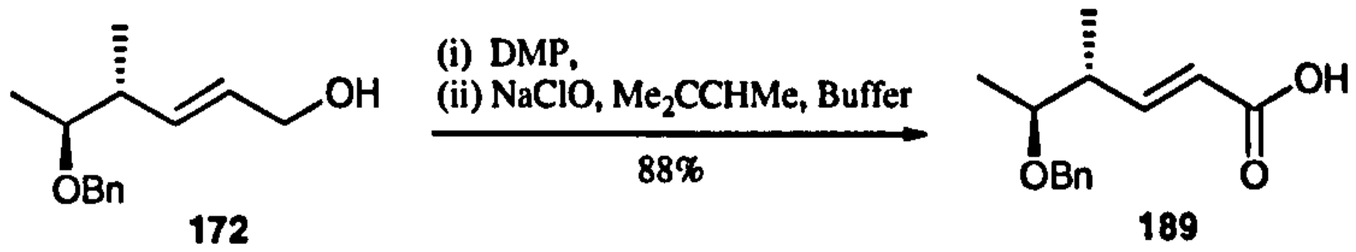
Scheme 77: Proposed mechanism of sodium trimethylsilanoate.[108]

Methyl ester **194** was prepared from benzyl ether **134** by cleavage of the auxiliary with DIBAL-H followed by Horner-Wadsworth-Emmons reaction using trimethylphosphonoacetate. Unfortunately, the latter reaction was not as high yielding, nor as selective for the *trans* ester as the analogous reaction to prepare ethyl ester **171**, affording 16% of the *cis* isomer and only 24% of the desired *trans* methyl ester **194**. Treatment of methyl ester **194** with sodium trimethylsilanoate however, proceeded in 85% yield affording the desired acid **189**.



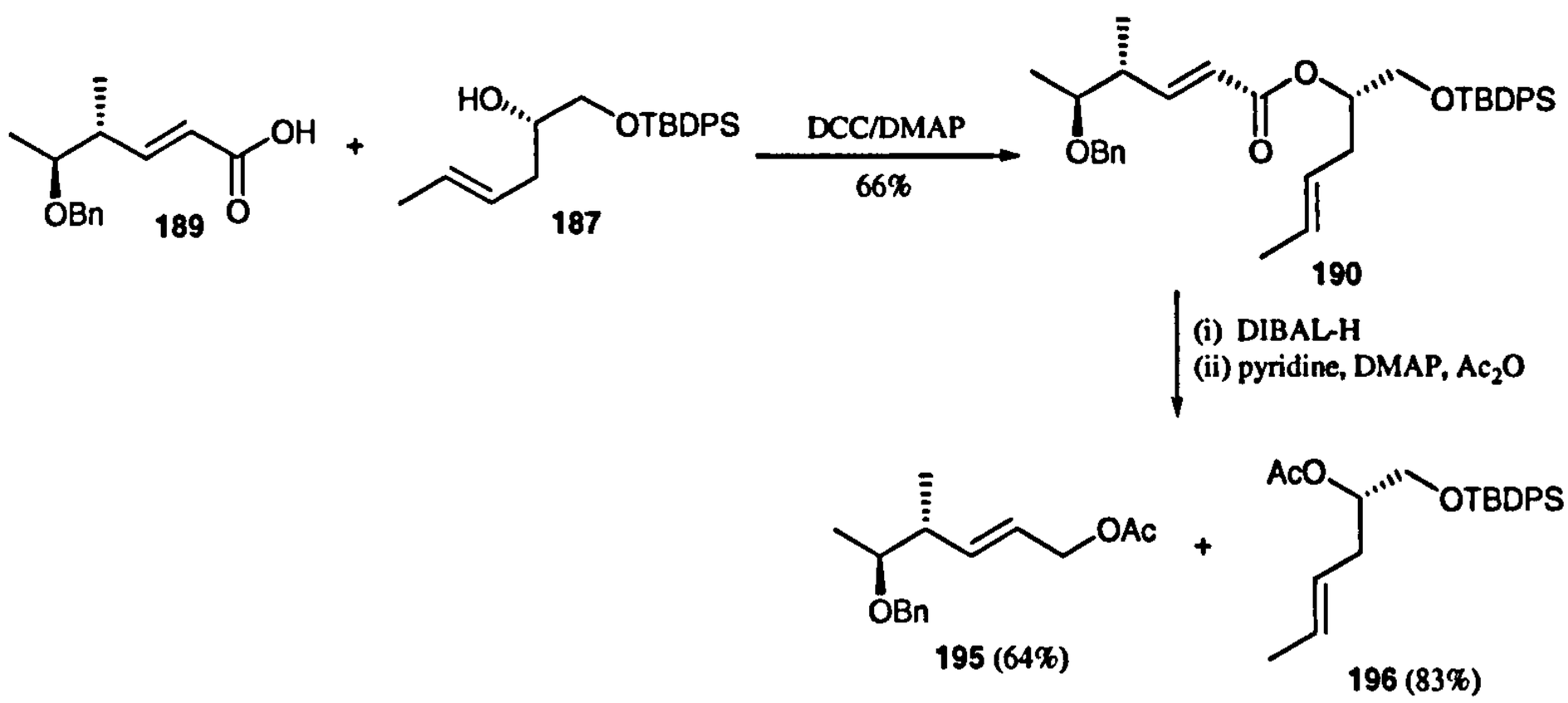
Scheme 78: Hydrolysis of methyl ester **194** using sodium trimethylsilanoate.

Due to the low yield obtained for methyl ester **194** in the previous route it was decided to access acid **189** from alcohol **172** *via* sequential Dess-Martin and Pinnick oxidations (Scheme 79). Although not as elegant an approach, as it effectively requires three steps to effect the desired ester to acid transformation, all three steps were fast, reliable and high yielding, furnishing acid **189** in 88% overall yield.



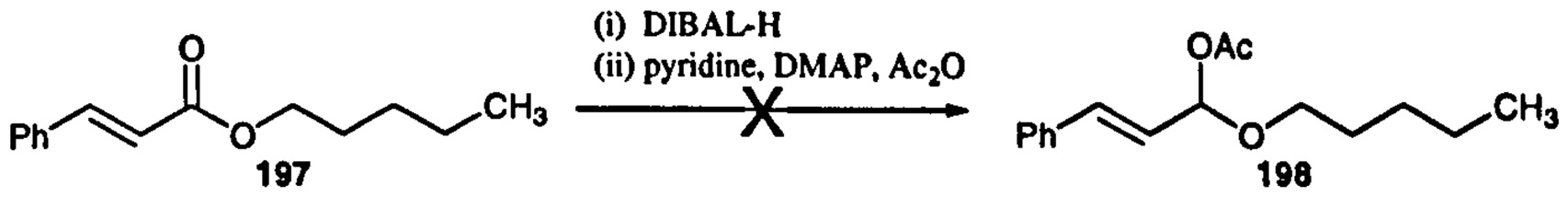
Scheme 79: Finalised route to acid **189.**

DCC/DMAP mediated esterification of homoallylic alcohol **187** and acid **189** proceeded in 66% yield to afford ester **190.** Unfortunately however, reductive acetylation did not give any of the desired α-acetoxy ether **191,** but resulted instead in high yields of acetates **195** and **196.**



Scheme 80: Formation of acetates **195** and **196.**

A more thorough literature search of Rychnovsky's papers revealed an extensive study of the conditions required for this reaction.[109] Each of the important reaction parameters were investigated including the number of equivalents of reagents, reaction times and temperature, quenching the reaction after the first step, solvent, substrates etc. Using their optimised conditions, most of the extensive range of substrates employed were converted to the corresponding α-acetoxy ethers in good to excellent yields. However, ester **197** failed to produce ether **198** under any of the conditions employed during the study (Scheme 81). Just as in the case of ester **190** exclusive overreduction was always observed. This was proposed to be due to the instability of the corresponding aluminium hemiacetal intermediate. Thus, we were forced to admit defeat with the segment-coupling approach. Due to time constraints we decided not to pursue this target further.



Scheme 81: Unsuccessful reductive acetylation of Kopecky and Rychnovsky.[109]

3.4 Conclusions

Mupiric acid **3** and its C-2 epimer **4** have been prepared using a cross metathesis approach. A 4:1 *trans/cis* mixture of each compound was obtained. The C-2 centre of **3** could thus be assigned as the *R* configuration by comparison of the ^{1}H NMR spectra of the synthetic acids **3** and **4** with that of the isolated metabolite.

A flexible route for the synthesis of mutant mupirocin metabolites lacking a 6-hydroxyl group has also been developed. This route centred on the proposed DH4 lactone **5**. Prins cyclisation to prepare the tetrahydropyran core was achieved (albeit in low yield) and problems such as labile protecting groups and bicycle formation were successfully overcome. Unfortunately, the problem of isomerisation due to side-reactions during this key step could not be resolved due to time constraints. If a means of speeding up the Prins cyclisation can be found in the future, this route should prove very useful for the preparation of novel mupirocin metabolites.

Chapter 4

Strobilurin A

4.1 The Strobilurins

Strobilurin A (or mucidin as it is also known), produced by *Strobilurus tenacellus* and *Oudemansiella mucida* (Figure 21) as well as several other basidiomycete and ascomycete fungi, is a potent fungicide.[110] It acts by inhibiting mitochondrial respiration, reversibly binding to the ubihydroquinone oxidation site (Qo) of the cytochrome bc_1 complex.[111]

Figure 21: *Strobilurus tenacellus*[112] and *Oudemansiella mucida*[113] - the mushroom producing fungi responsible for strobilurin A biosynthesis.

The potential of strobilurin A as a candidate for agrochemical development was recognised soon after its discovery in 1976.[114] Its high antifungal activity in laboratory tests coupled with its "novel" mode of action and low toxicity towards mammalian cells were favourable features. Another advantage of using a natural product as the basis of an agrochemical lies in its ability to biodegrade, posing no lasting threat to the environment.

During greenhouse testing, however, strobilurin A was found to be unstable. This lack of stability was attributed to the triene system of the molecule which was thought to be subject to photolytic and/or oxidative breakdown.[114] This led to widespread interest in the structural modification of strobilurin A as several companies competed to develop the perfect synthetic analogue. Many synthetic analogues of strobilurin A have since been developed,[115] all of which exploit the novel mode of action of the parent compound (Figure 22). These were originally marketed commercially by Zeneca, BASF, Shionogi and Novartis under a variety of trade names.

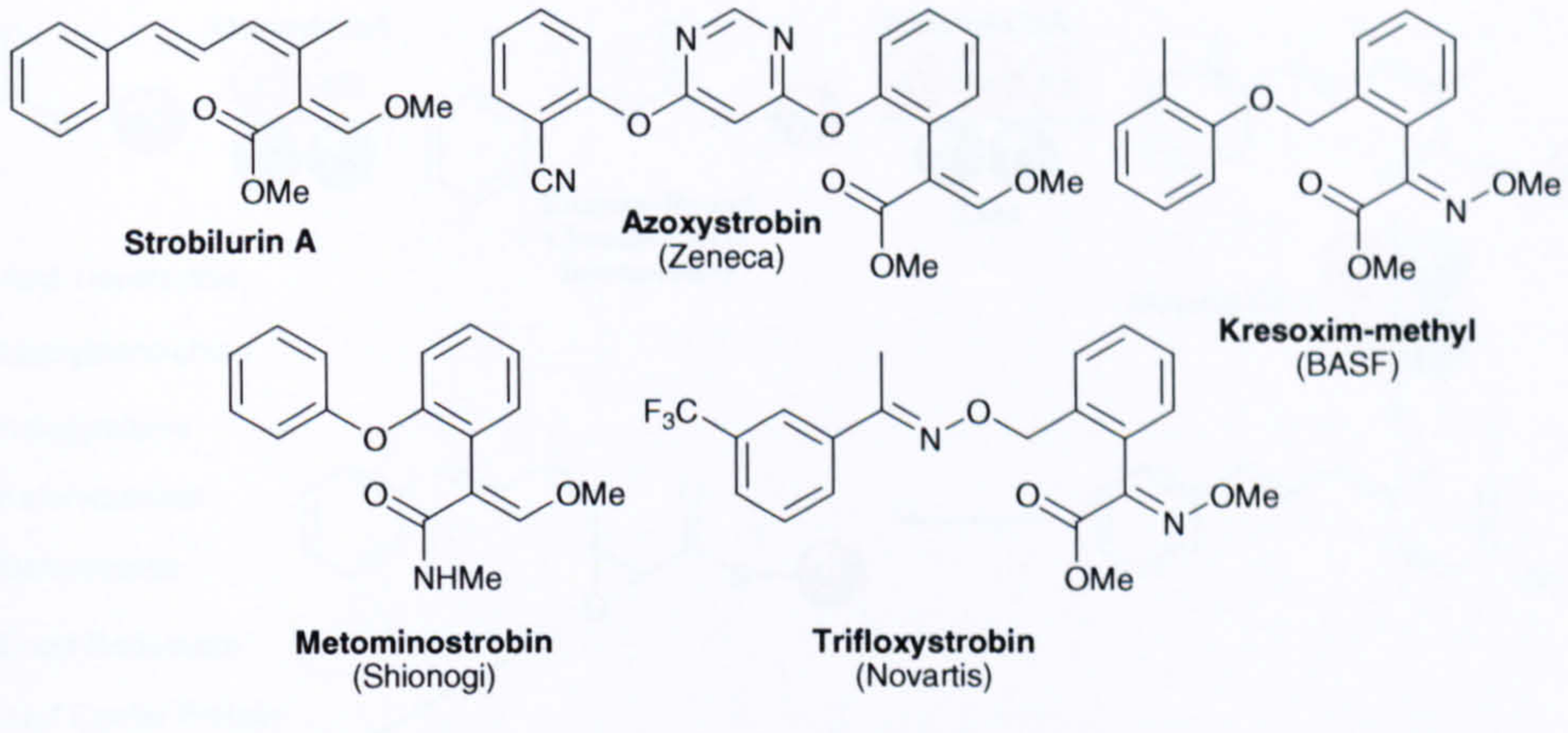


Figure 22: Agrochemicals based on the structure of strobilurin A.

Many feeding studies have been performed on the fungi *Oudemansiella mucida*[116] and *Strobilurus tenacellus*[117] in an effort to elucidate the biosynthetic pathway to strobilurin A. These are summarised in Figure 23.

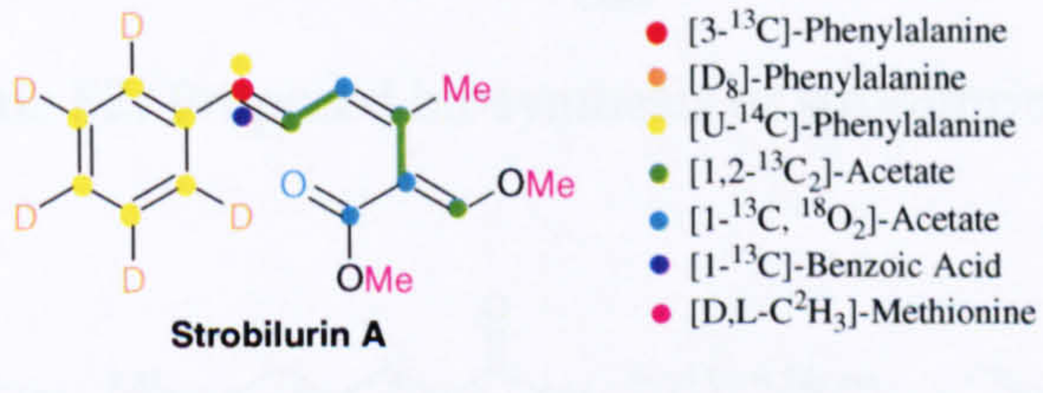
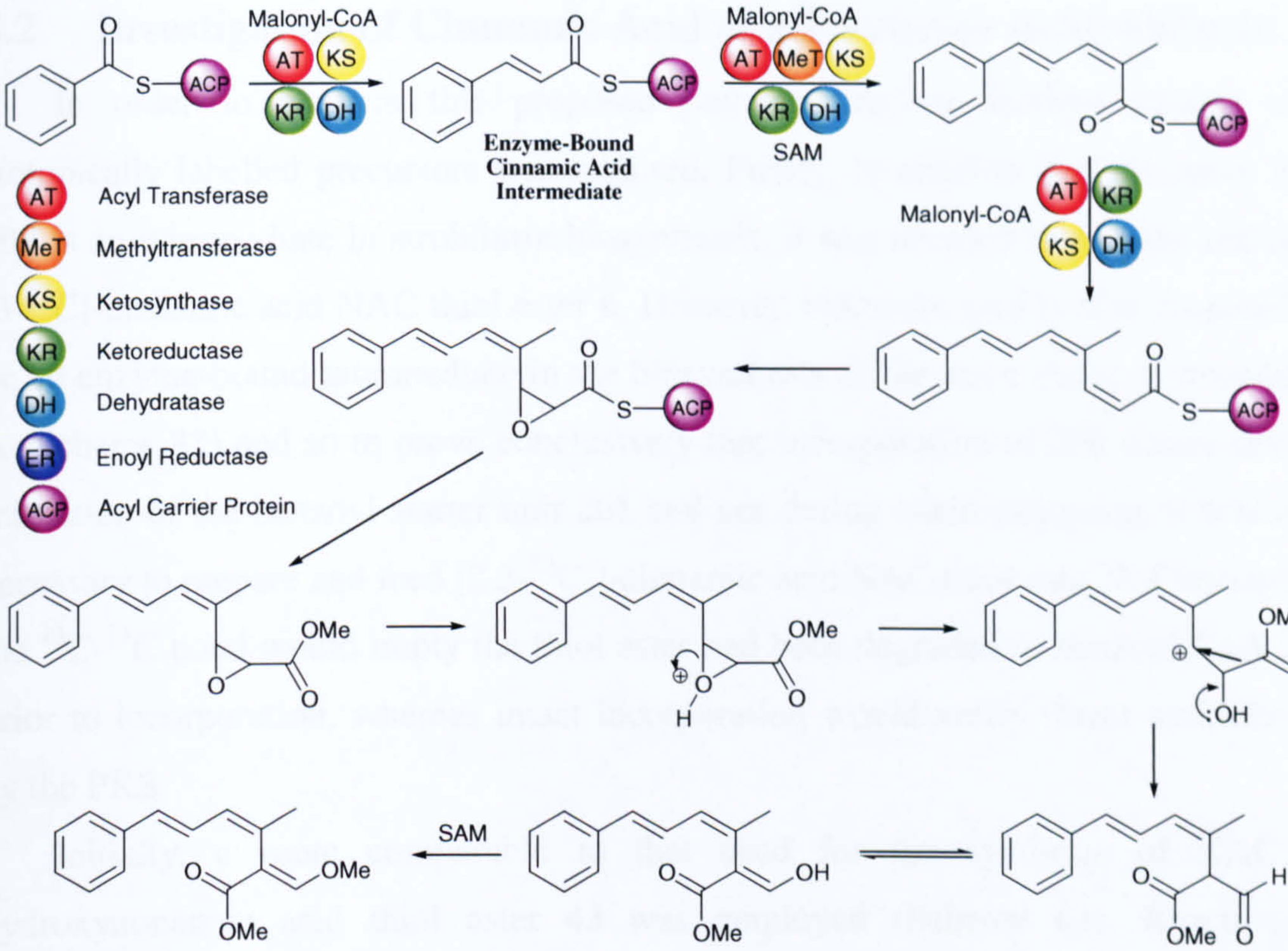
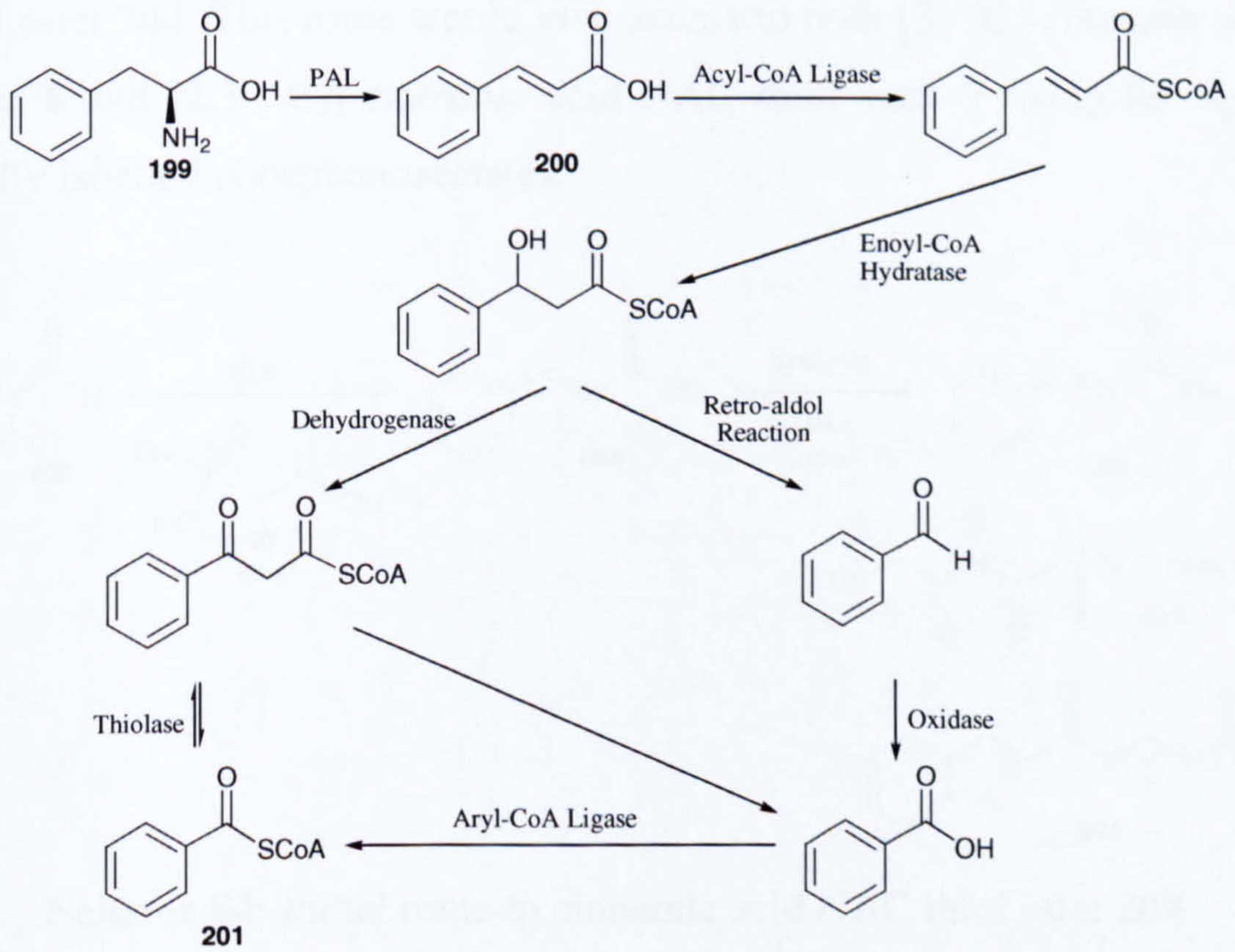


Figure 23: Labelling pattern of strobilurin A from acetate, benzoic acid, phenylalanine and methionine precursors.[116,117]

These studies have demonstrated that strobilurin A is of mixed biosynthetic origin. The aliphatic portion is acetate derived and is likely to follow a polyketide biosynthetic pathway followed by a pinacol-like rearrangement (Scheme 82),[118] whereas the benzyl fragment appears to be synthesized from phenylalanine **199**, the latter being converted to benzoyl-CoA **201** prior to assembly. A possible route for this conversion *via* cinnamic acid **200** has been proposed by Zeeck and Thormann (Scheme 83).[117]



Scheme 82: Proposed biosynthesis of strobilurin A.[118]

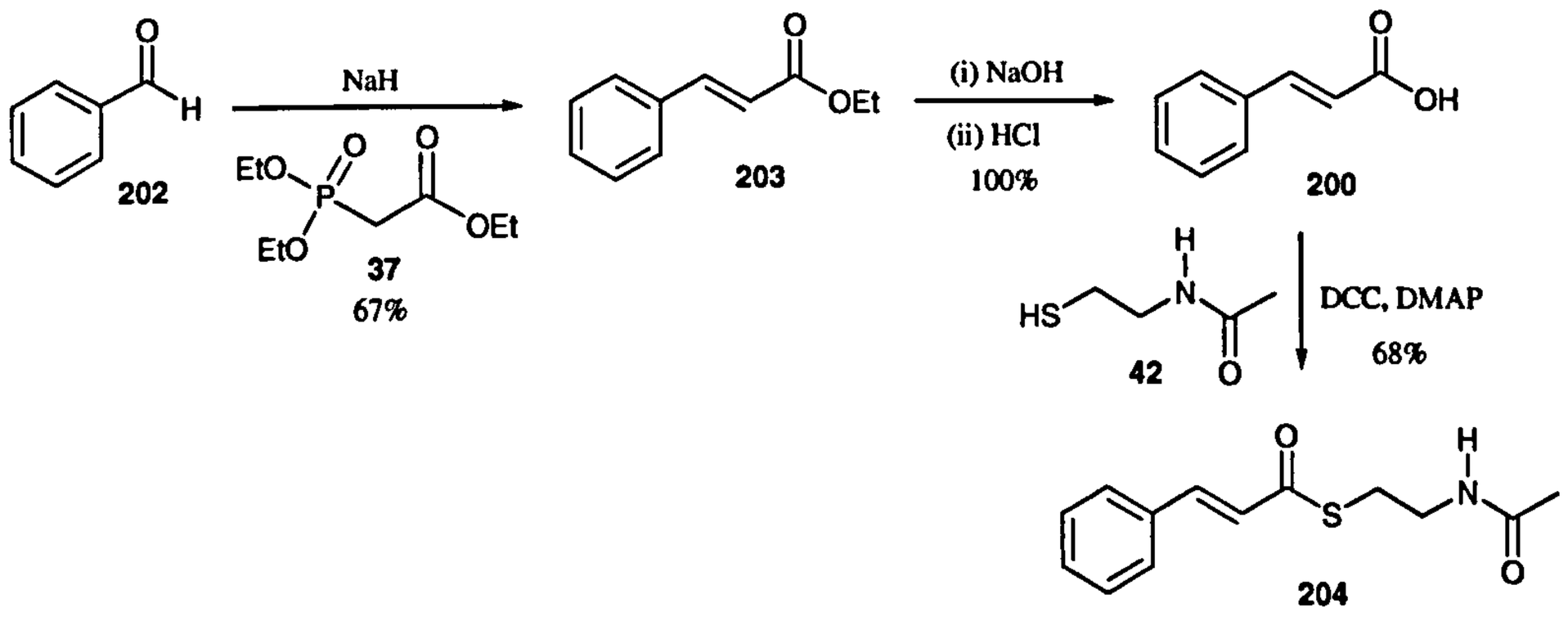


Scheme 83: Proposed biosynthesis of benzoyl-CoA starter unit **201**.[117]

4.2 Investigation of Cinnamic Acid as a Precursor to Strobilurin A

In order to confirm this proposed pathway, further feeding studies with isotopically labelled precursors was required. Firstly, to confirm that cinnamic acid **200** is an intermediate in strobilurin biosynthesis, it was decided to prepare and feed [3-^{13}C]-cinnamic acid NAC thiol ester **6**. However, cinnamic acid is also proposed to be an enzyme-bound intermediate in the biosynthesis of the main chain of strobilurin A (Scheme 82) and so to prove conclusively that incorporation of **200** occurs during formation of the benzoyl starter unit **201** and not during chain extension, it was also necessary to prepare and feed [2,3-$^{13}C_2$]-cinnamic acid NAC thiol ester **7**. Cleavage of the ^{13}C-^{13}C bond would imply the thiol ester had been degraded to benzoyl-CoA **201** prior to incorporation, whereas intact incorporation would verify direct assimilation by the PKS.

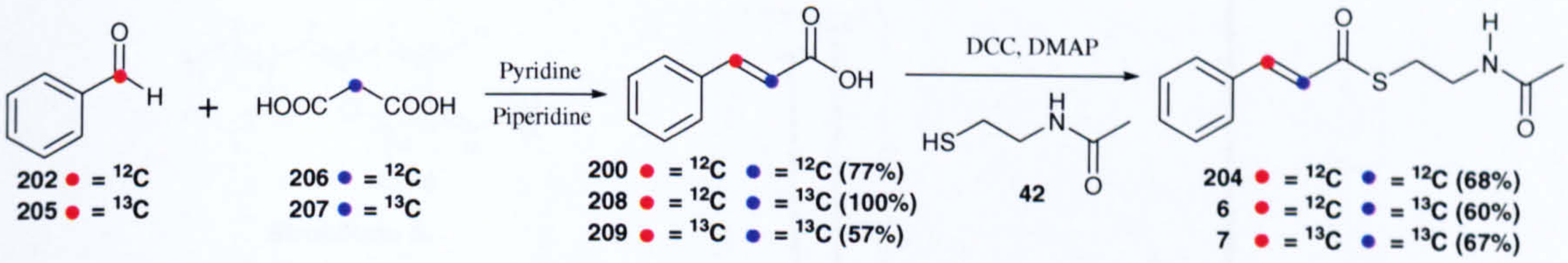
Initially, a route comparable to that used for the synthesis of NAC 9-hydroxynonanoic acid thiol ester **43** was employed (Scheme 84). Reaction of benzaldehyde **202** with triethyl phosphonoacetate **37** furnished ethyl ester **203** in 67% yield. Subsequent hydrolysis and coupling reactions gave the required NAC cinnamic acid thiol ester **204**. This route would give access to both [3-^{13}C]-cinnamic acid NAC thiol ester **6** and [2,3-$^{13}C_2$]-cinnamic acid NAC thiol ester **7** using the appropriate isotopically labelled phosphonoacetates.



Scheme 84: Initial route to cinnamic acid NAC thiol ester **204**.

On reflection, however, it was decided that a simple two step procedure incorporating features of two documented methods for the synthesis of cinnamic acid and cinnamic acid NAC thiol ester[119,120] may be more efficient. The approach was first optimised using unlabelled material (Scheme 85). Following the method of

Augustyniak *et al.*,[119] benzaldehyde **202** and malonic acid **206** in the presence of base underwent a Knoevenagel reaction, affording cinnamic acid **200** in 77% yield. This was then converted to the NAC thiol ester **204** in 68% yield using *N*-acetylcysteamine **42**, DCC and DMAP.



Scheme 85: Synthetic route to cinnamic acid NAC thiol esters **6**, **7** and **204**.

[3-^{13}C]-cinnamic acid NAC thiol ester **6** was similarly prepared from [carbonyl-^{13}C]-benzaldehyde **205** in 60% overall yield. Interestingly, a by-product was observed during the synthesis of thiol ester **6** which did not arise previously with the unlabelled material. Although this compound was not isolated, crude ^{1}H NMR data suggested it was the addition product observed by Bjorklund and Leete[120] (Figure 24).

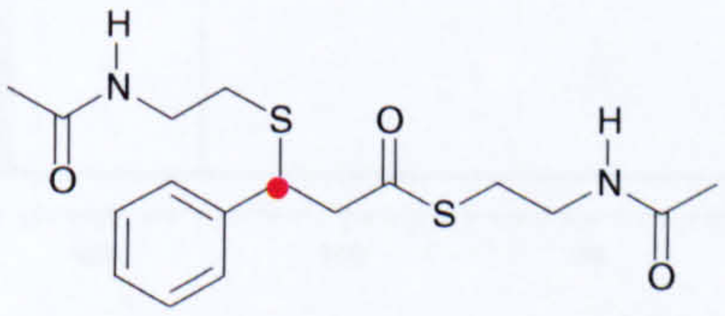


Figure 24: Addition by-product observed by Bjorklund and Leete.[120]

[2,3-$^{13}C_2$]-cinnamic acid NAC thiol ester **7** was then prepared from [carbonyl-^{13}C]-benzaldehyde **205** and [2-^{13}C]-malonic acid **207** in 57% overall yield. Again the addition by-product was observed but to a lesser extent than in the previous experiment. A small quantity of *N*,*S*-diacetylcysteamine was also observed which could not initially be separated from the product by column chromatography or recrystallisation (an analytical sample was later purified by column chromatography using a methanol/ethyl acetate solvent system). However, as it was not expected to cause a problem during feeding studies, the mixture was fed without further purification.

Thiol esters **6** and **7** were then sent to Universität Göttingen where feeding studies were performed by Zeeck and Thormann. Having fed approximately 150 mg of the labelled precursor **6** to *Strobilurus tenacellus*, strobilurin A was isolated,

analysed by ^{13}C NMR and compared to unlabelled material (Figure 25). A 17 fold enhancement (compared with the natural abundance spectrum) was observed for the ^{13}C signal at 131.9 ppm, assigned to C-7, confirming that cinnamic acid is a likely precursor to strobilurin A (Figure 26).

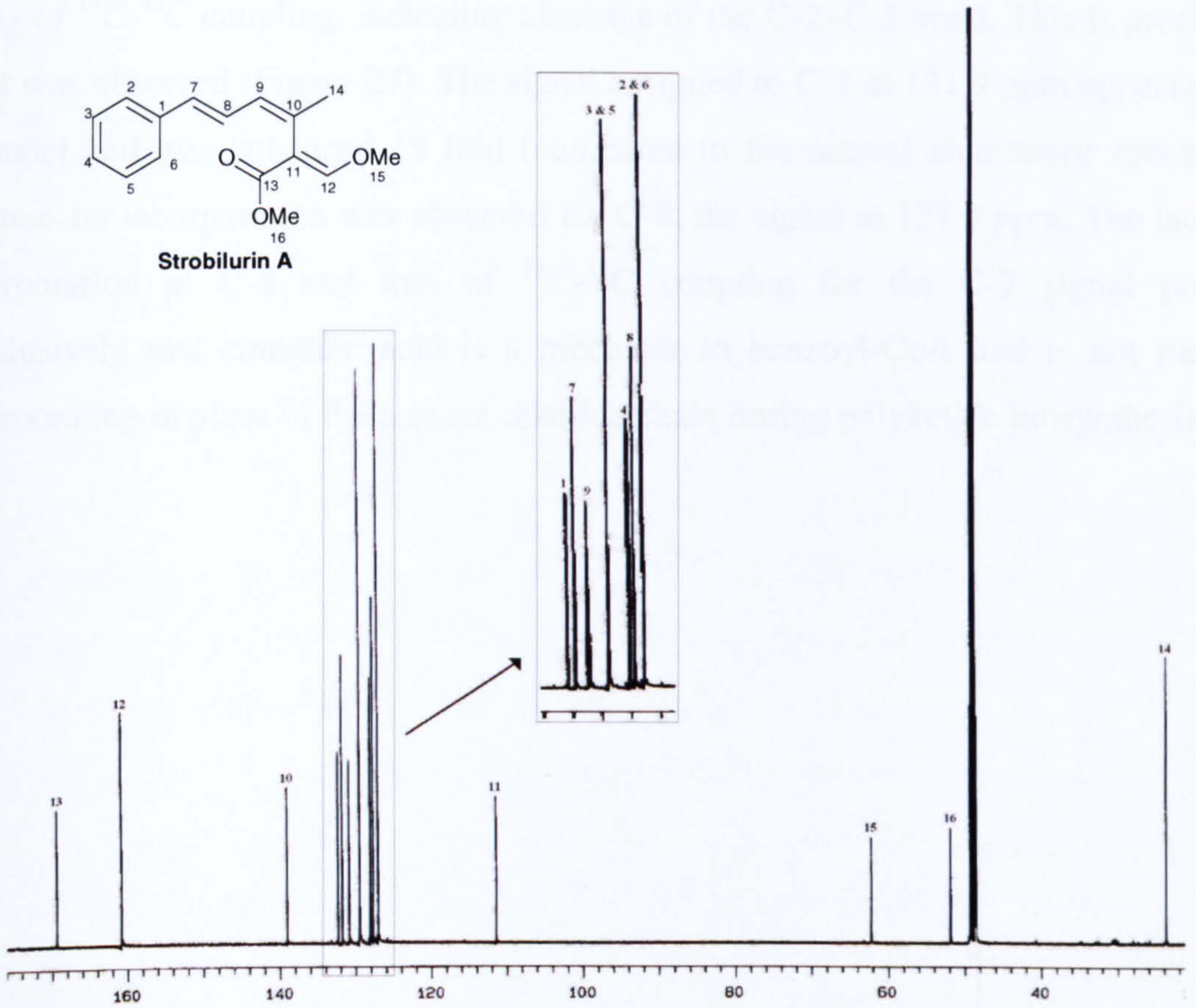


Figure 25: ^{13}C NMR spectrum of strobilurin A in CD_3OD, courtesy of Zeeck and Thormann.

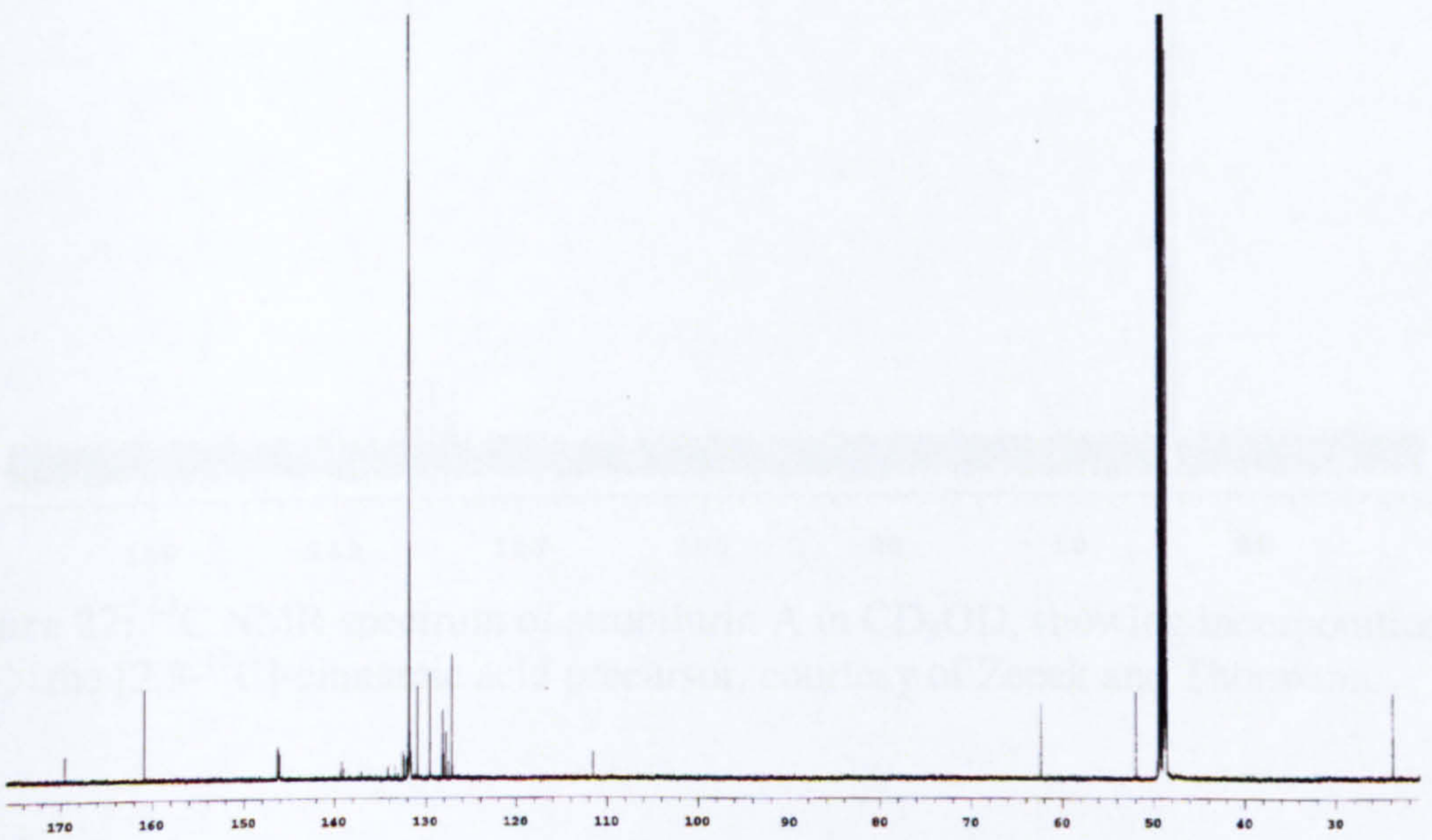


Figure 26: ^{13}C NMR spectrum of strobilurin A in CD_3OD, showing incorporation of the [3-^{13}C]-cinnamic acid precursor, courtesy of Zeeck and Thormann.

[2,3-$^{13}C_2$]-cinnamic acid NAC thiol ester **7** (100 mg) was subsequently fed to the fungus and again the isolated product was analysed by ^{13}C NMR. Assuming the proposed biosynthesis of the benzoyl-CoA starter unit is correct, ^{13}C incorporation at the C-7 position but not at the C-8 position would be expected. We would also predict a loss of ^{13}C-^{13}C coupling, indicating cleavage of the C-2–C-3 bond. This is precisely what was observed (Figure 27). The signal assigned to C-7 at 131.9 ppm appeared as a singlet and was enhanced 13 fold (compared to the natural abundance spectrum) whereas no incorporation was observed for C-8, the signal at 127.9 ppm. The lack of incorporation at C-8 and loss of ^{13}C-^{13}C coupling for the C-7 signal proves conclusively that cinnamic acid is a precursor to benzoyl-CoA and is not merely incorporating in place of the nascent diketide chain during polyketide biosynthesis.

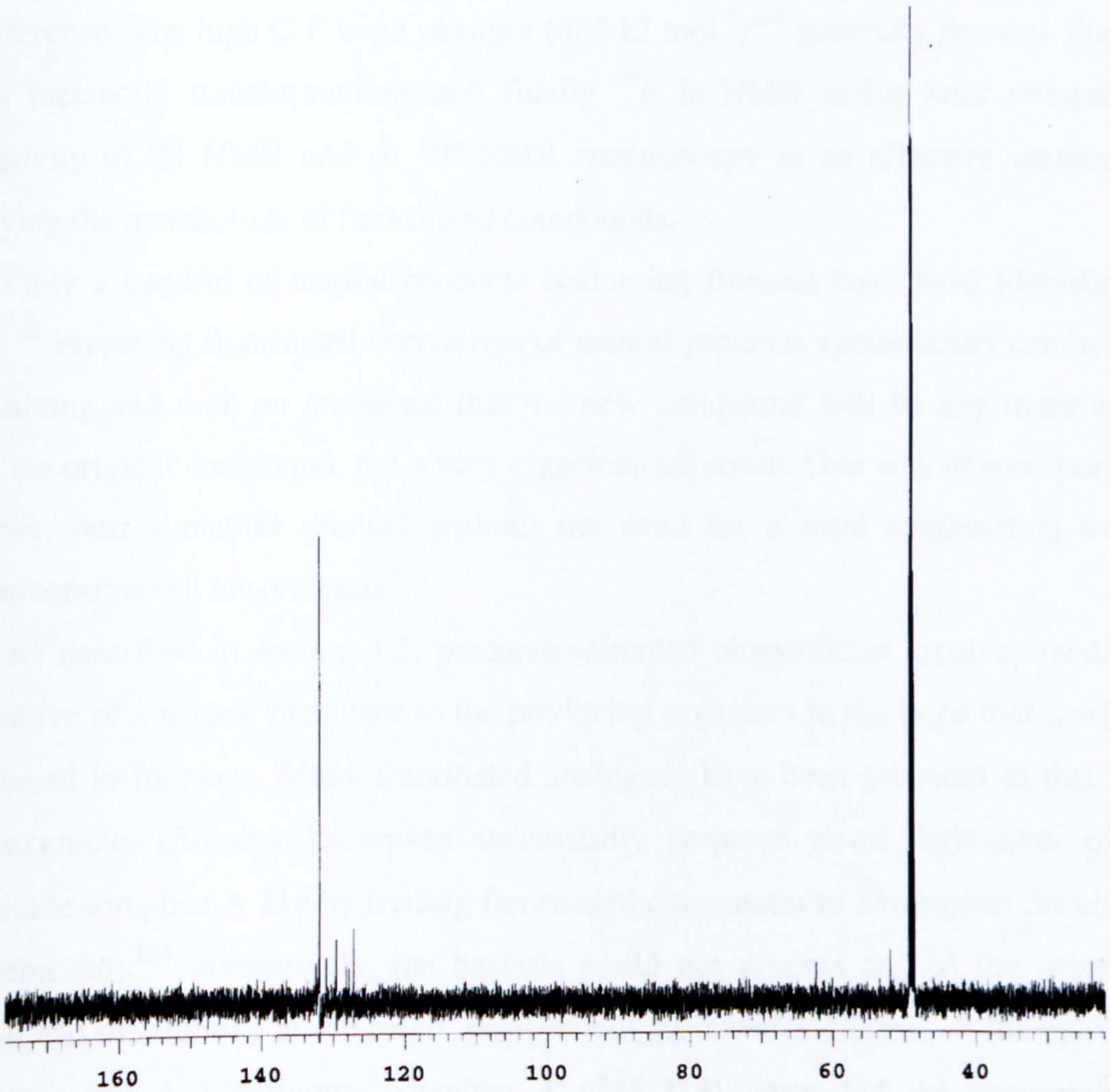


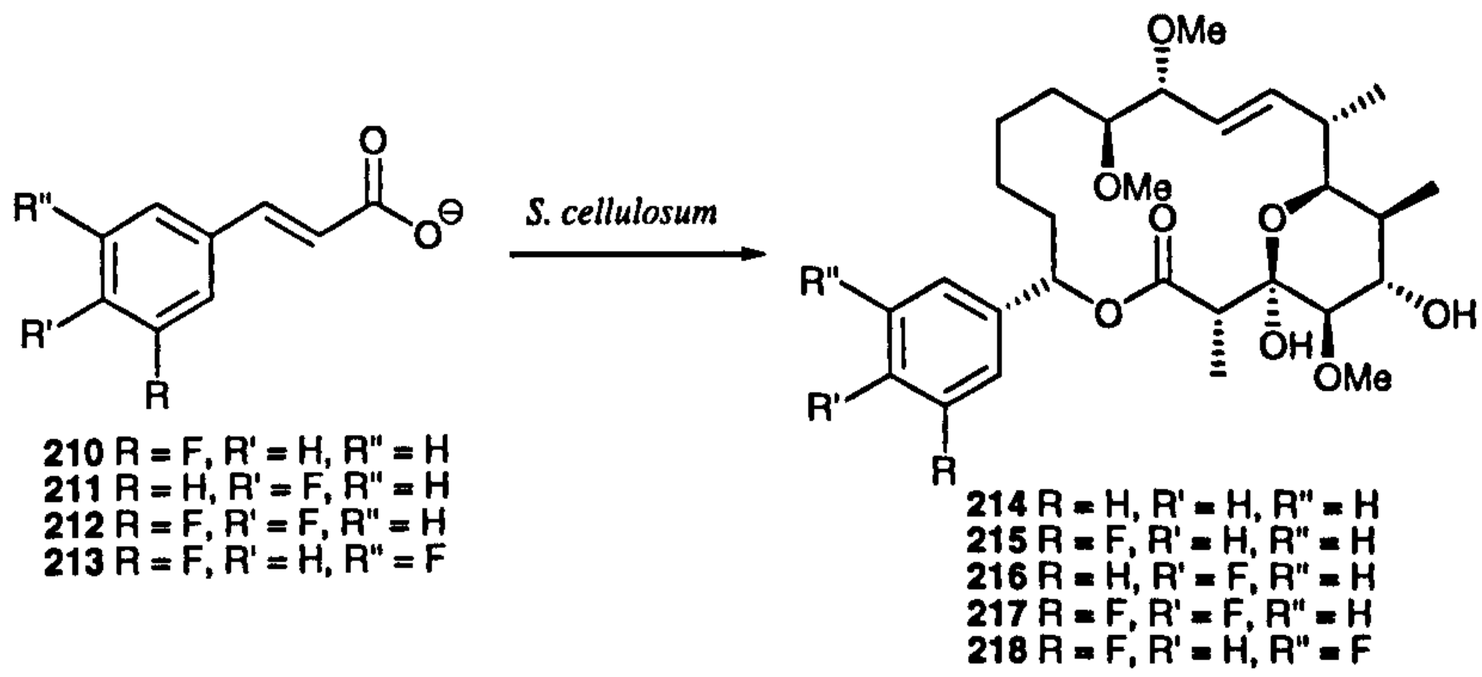
Figure 27: ^{13}C NMR spectrum of strobilurin A in CD_3OD, showing incorporation of the [2,3-^{13}C]-cinnamic acid precursor, courtesy of Zeeck and Thormann.

4.3 Investigation of the Role of *p*-Fluorocinnamic Acid in the Precursor-Directed Biosynthesis of *p*-Fluorostrobilurin A

Organofluorine compounds are of great medicinal value[121] and a significant number of important man-made drugs, including prozac® (fluoxetine), mefloquin® (fluconazole) and cypro® (cyprofloxacin) contain fluorinated active pharmaceutical ingrediants. The replacement of hydrogen with fluorine results in a strongly polarised C-F bond, which can alter the acidity of neighbouring functional groups[122] and fluorine can also function as a hydrogen bond acceptor.[123] This affects the substrate-enzyme binding of the molecule and ultimately its bioactivity. For several reasons fluorine is also a useful biomarker. Firstly, it is similar in size to a hydrogen atom (1.35 versus 1.20 Å)[122] and can therefore replace hydrogen without notable steric interference. The high C-F bond strength (485 kJ mol^{-1})[124] generally protects fluorine from metabolic transformations and finally ^{19}F is NMR active with comparable sensitivity to ^{1}H NMR and so ^{19}F NMR spectroscopy is an effective method for studying the metabolism of fluorinated compounds.

Only a handful of natural products containing fluorine have been identified to date.[122] Preparing fluorinated derivatives of natural products synthetically can be time consuming and with no guarantee that the new compound will be any more active than the original compound, not a very sagacious approach. One way of incorporating fluorine into a natural product without the need for a total synthesis is to use precursor-directed biosynthesis.

As described in section 1.3, precursor-directed biosynthesis involves feeding a derivative of a natural precursor to the producing organism in the hope that it will be processed in its place. Many fluorinated analogues have been prepared in this way. For example, Hill and Thompson successfully prepared novel derivatives of the fungicide soraphen A **214** by feeding fluorinated cinnamates to *Sorangium cellulosum* (Scheme 86).[125] Interestingly, the bacteria could not process any of the substrates which had the fluorine at the *ortho*- position but produced 3′-fluoro-, 4′-fluoro-, 3′,4′-difluoro- and 4’,5’-difluoro- soraphen A (**215-218**) when fed the corresponding cinnamates (**210-213**).

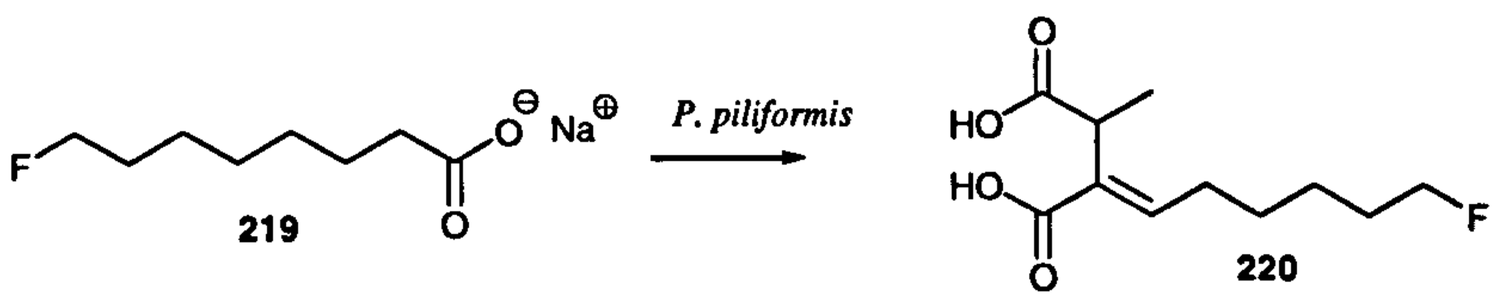


210 R = F, R' = H, R" = H
211 R = H, R' = F, R" = H
212 R = F, R' = F, R" = H
213 R = F, R' = H, R" = F

214 R = H, R' = H, R" = H
215 R = F, R' = H, R" = H
216 R = H, R' = F, R" = H
217 R = F, R' = F, R" = H
218 R = F, R' = H, R" = F

Scheme 86: Precursor-directed biosynthesis of fluorinated soraphen A derivatives **215-218** in *S. cellulosum*.[125]

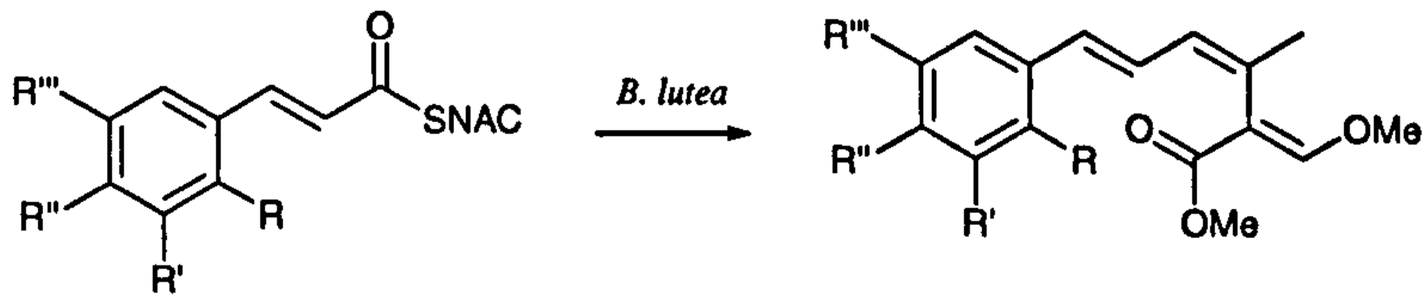
O'Hagan and co-workers[126] studied the incorporation of sodium 8-fluorooctanoate **219** into piliformic acid by *Poronia piliformis* (Scheme 87) and found it incorporated to almost the same extent as the natural octanoate precursor, furnishing the novel 9-fluoropiliformate **220**.



219 **220**

Scheme 87: Precursor-directed biosynthesis of fluorinated piliformic acid **220** in *P. piliformis*.[126]

Soares-Sello conducted several precursor-directed biosynthetic studies on the strobilurins during the course of her PhD.[118] Working with *Bolinea lutea*, she fed a variety of fluorinated precursor analogues, including free acids, benzoic acid NAC thiol esters, cinnamic acid NAC thiol esters and phenylalanines, and determined that the cinnamic acid NAC thiol esters (**221-226**) afforded the best yields and levels of incorporation (Scheme 88). However, although she could detect the possible presence of 2-fluoro-, 3-fluoro-, 4-fluoro-, 3,4-difluoro-, 3,5-difluoro- and 3-fluoro-4-methyl-strobilurin A analogues (**227-232**) in the fungal extracts, she was unable to separate them from the natural compound by preparative T.L.C.



221 R = F, R' = H, R'' = H, R''' = H
222 R = H, R' = F, R'' = H, R''' = H
223 R = H, R' = H, R'' = F, R''' = H
224 R = H, R' = F, R'' = F, R''' = H
225 R = H, R' = F, R'' = H, R''' = F
226 R = H, R' = F, R'' = Me, R''' = H

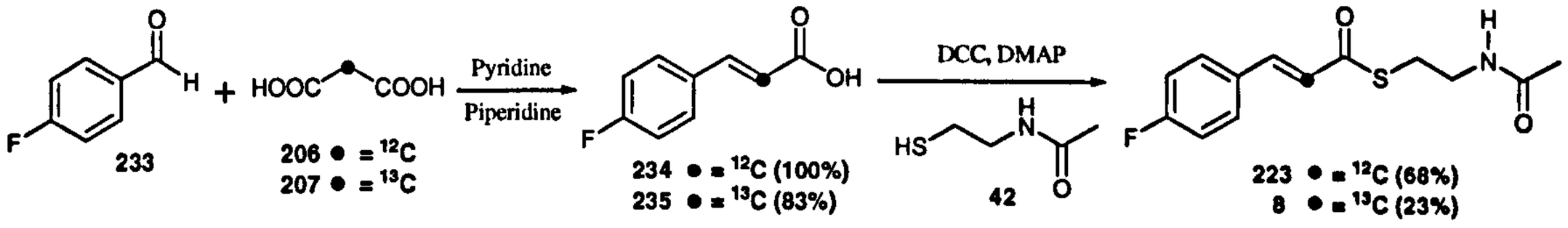
227 R = F, R' = H, R'' = H, R''' = H
228 R = H, R' = F, R'' = H, R''' = H
229 R = H, R' = H, R'' = F, R''' = H
230 R = H, R' = F, R'' = F, R''' = H
231 R = H, R' = F, R'' = H, R''' = F
232 R = H, R' = F, R'' = Me, R''' = H

Scheme 88: Precursor-directed biosynthesis of fluorinated strobilurins in *B. lutea*.[118]

Thormann also prepared a variety of fluorinated strobilurins.[117] By feeding fluorinated benzoic acid NAC thiol esters and cinnamic acid NAC thiol esters (prepared in Bristol) to *Strobilurus tenacellus*, he was able to isolate and purify the fluorinated strobilurin analogues by HPLC. However, in contrast to the work of Soares-Sello, Thormann found that the benzoic acid NAC thiol esters gave higher incorporation than the cinnamic acid NAC thiol esters.

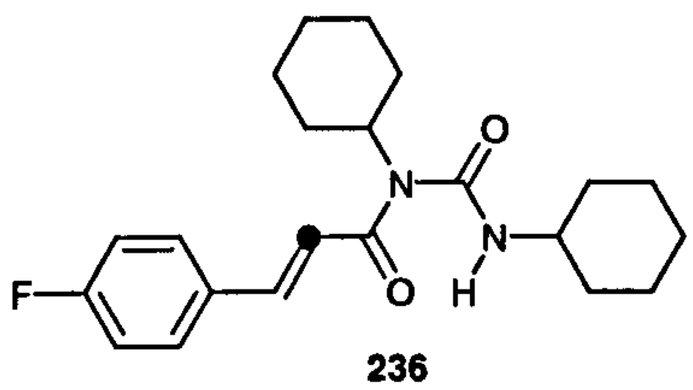
As discussed in section 1.1.1 the level of incorporation of one isotopically labelled precursor compared to another may possibly be an indication as to their relative positions in a biosynthetic pathway. Although other factors play a role, the higher incorporation of benzoic acid NAC thiol esters in Thormann's experiments might indicate that benzoic acid occurs later in the biosynthetic pathway than cinnamic acid. This is in agreement with cinnamic acid degrading to benzoyl-CoA prior to assembly. Equally the work of Soares-Sello might indicate that cinnamic acid occurs later in the biosynthetic pathway than benzoic acid, incorporating during chain assembly. To settle the debate (at least in the case of *Strobilurus tenacellus*) it was decided to prepare [2-^{13}C]-*p*-fluorocinnamic acid NAC thiol ester **8** for a feeding study. As before, intact incorporation of both biomarkers would imply incorporation of the precursor during polyketide synthesis and not during starter unit production.

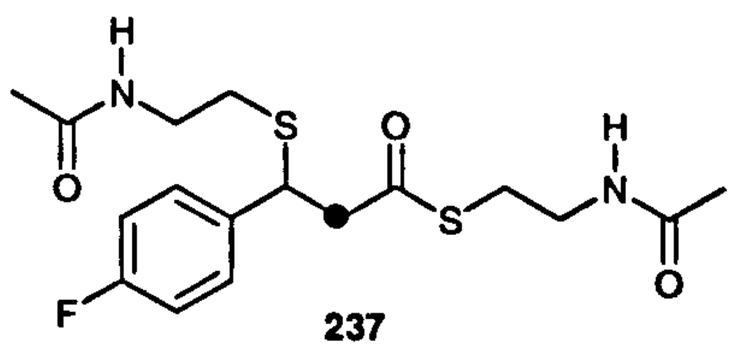
p-fluorocinnamic acid NAC thiol ester **223** was prepared as shown in Scheme 89. Knoevenagel condensation of *p*-fluorobenzaldehyde **233** with malonic acid **206** furnished *p*-fluorocinnamic acid **234** in 100% yield. DCC/DMAP mediated coupling with *N*-acetylcysteamine **42** afforded the corresponding thiol ester **223** in 68% yield. Purification of the thiol ester proved challenging due to the formation of *N,S*-diacetylcysteamine *in situ* which virtually co-ran with thiol ester **223**. Increasing the polarity of the eluent by the addition of methanol however, led to improved separation and easier purification of the desired compound.

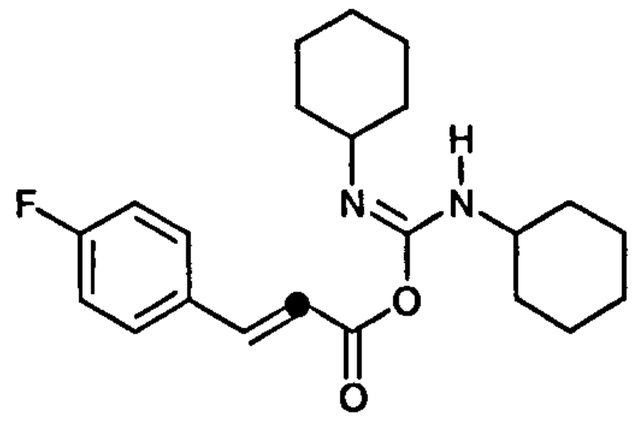


Scheme 89: Preparation of thiol esters **223** and **8**.

The same route was followed to prepare thiol ester **8** from [2-^{13}C]-malonic acid **207** in 21% overall yield. This low yield was due to the formation of two products which had not been observed previously. The first was the *N*-acyl urea **236**. Precedence for this type of compound is seen in the work of Holmberg[127] and Keck.[128] It is proposed that this stable compound is formed by rearrangement of the *O*-acyl urea **238** which is the first reaction intermediate in the coupling process. Keck implies that dilute solutions are more prone to this rearrangement and both groups found the addition of strong acid catalysts to be beneficial in counteracting the problem. The second by-product is the addition product **237** analogous to that observed by Bjorklund and Leete.[120]


236


237


238

Thiol ester **8** was sent to Germany where Zeeck and Thormann conducted a feeding study with *Strobilurus tenacellus*. Unfortunately, no ^{13}C or ^{19}F incorporation was observed in the isolated product and so no conclusions could be drawn from this experiment.

4.4 Conclusions

[3-^{13}C]-Cinnamic acid thiol ester **6** and [2,3-$^{13}C_2$]-cinnamic acid thiol ester **7** were prepared in two steps from isotopically labelled benzaldehyde and malonic acid and fed to *S. tenacellus* by Zeeck and Thormann. Thiol ester **6** showed specific incorporation at C-7 of strobilurin A, indicating that cinnamic acid is a likely precursor to strobilurin A. Thiol ester **7** also incorporated solely at the C-7 position with a loss of ^{13}C-^{13}C coupling, indicating cleavage of the C-2–C-3 of the precursor prior to incorporation.

[2-^{13}C]-*p*-Fluorocinnamic acid thiol ester **8** was analogously prepared and fed to *S. tenacellus*. No ^{13}C or ^{19}F incorporation was observed indicating that the attempted precursor-directed biosynthesis had failed.

Chapter 5

Alternapyrone

5.1 Phytotoxins Produced by *Alternaria solani*

Alternaria solani, a causal fungus of early blight disease in tomato and potato plants (as well as other solanaceous plants),[129] is an internationally significant phytopathogen due to its worldwide distribution and the economic importance of the crops which it affects.[130]

Early blight is often more prevalent in older, senescing tissues and particularly in plants that have been predisposed by injury, poor nutrition, insect damage, heavy fruit load or another type of stress.[129] Initial infection emerges as dark brown lesions on the surface of the leaves, fruit and tubers.[129,131] As the fungus penetrates, assimilating the nutrients from the dead plant cells, the spots enlarge, developing concentric rings of raised and depressed necrotic tissue where new spores are produced (Figure 28). Eventually, the leaves dry up or fall off and the fruit and tubers become soft and shrivelled.

Figure 28: Early blight symptoms of *Alternaria solani* in potato leaves,[132] tomato fruit[133] and potatoes.[132]

A. solani produces numerous non-host specific toxins[134] (some of which are host specific-like) which intensify the symptoms of the disease, helping to degrade and kill the host plant cells. These toxins often diffuse into the surrounding tissues causing a yellow chlorotic halo to form around the necrotic spots (most evident in the leaves).

Many of the toxins produced by *A. solani* are polyketide in origin, displaying high degrees of structural diverity (Figure 29). Alternaric acid, isolated by Brian *et al.* in 1949,[135] was found to have remarkably specific antifungal activity, inhibiting the germination of *Absidia glauca*, *Myrothecium verrucaria* and *Stachybotrys atra* spores but not those of fungi such as *Botyrytis allii*, *Fusarium coeruleum* and *Penicillium digitatum*. It was also found to be a host specific-like toxin contributing to disease development in the plant host by the fungus. It does this by altering the plasma morphological and physiological characteristics of membranes near the

plasmodesmata (responsible for the transportation of molecules from one cell wall to another and intercellular signalling), thereby causing a permeability change that leads to leakage of electrolytes (mostly Mg^{2+}) from the plant cells.[136] Since intact plasma membranes are necessary for the maintenance of ionic and metabolic gradients, the modifications caused by alternaric acid effectively kills the plant cells, allowing the fungus to infiltrate.

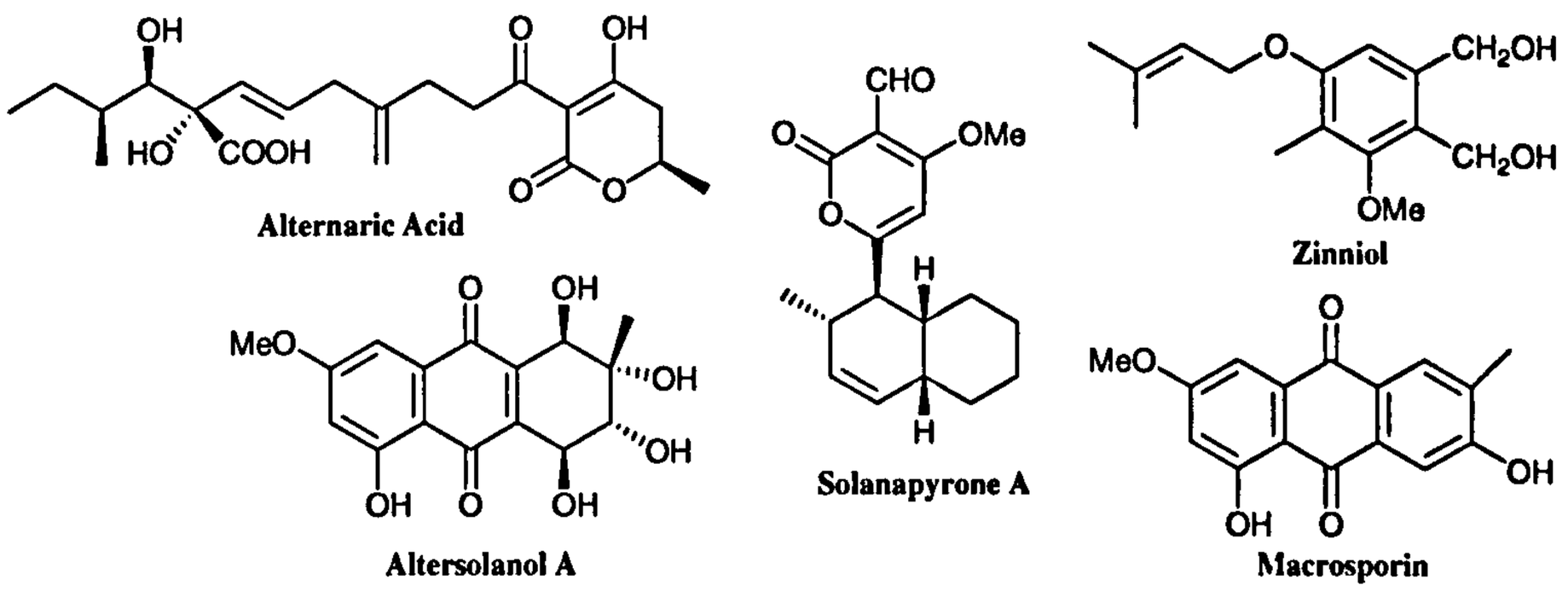


Figure 29: Some of the many polyketide metabolites produced by the fungus *Alternaria solani.*

The biochemical mode of action of the phytotoxin solanapyrone A, isolated in 1983 by Ichihara *et al.*,[137] is as yet unknown. However, it has been shown to selectively inhibit the activities of mammalian DNA polymerase β and λ *in vitro*, binding to the *N*-terminal of the protein.[138] Because of this polymerase species-specificity, solanapyrone A is currently being investigated as a potential cancer chemotherapy agent. Another interesting feature of this polyketide is the enzymatically catalysed Diels-Alder reaction by which it is produced.[139,140] The solanapyrones were the first group of natural products to have been proven to contain a Diels-Alderase domain within their synthase.

The phytotoxic tetraketide zinniol, discovered in 1968 by Starratt,[141] is produced by several species of *Alternaria* fungi, including *A. zinniae*,[141] *A. dauci*,[142] *A. tagetica*,[143] *A. carthami*[144] and *A. solani*.[145,146] It was found to bind to the protoplasts (cell from which the outer cell wall or membrane has been removed) and membranes of susceptible plants.[147] Receptor occupancy results in the stimulation of calcium uptake by the effected cells and also partially inhibits the effects of calcium-channel blockers. Calcium is a secondary messenger and is known to control various processes in the plant including intracellular organisation and various enzyme

activities.[148,149] A non-regulated increase in cellular calcium therefore may have a lethal effect on plant cells.

The red pigment altersolanol A, isolated by Stoessl in 1967,[150] was found to have antibiotic[151] as well as phytotoxic[152] activity. In both instances, activity was attributed to stimulation of NADH oxidase by altersolanol A which also acts as an electron acceptor in the respiratory chain. This increase in respiration results in the excessive generation of reactive oxygen species (such as O_2^-, H_2O_2 and $^{\cdot}OH$) which in turn causes lipid peroxidation, DNA damage and protein oxidation.[153]

Macrosporin, first isolated in 1957 by Suemitsu *et al.* from *Macrosporium porri*,[154] was also found to be a metabolite of *A. solani*.[155] This yellow-orange phytotoxin pigment was found to be a biosynthetic derivative of altersolanol A.[145,146]

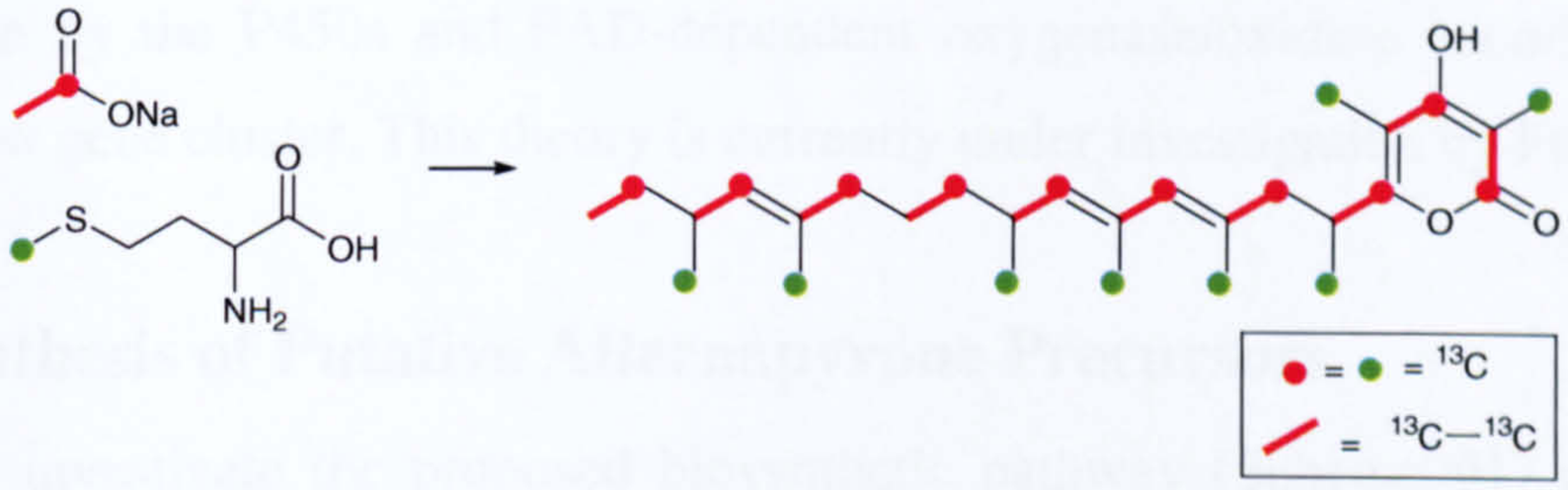
5.2 Discovery of Alternapyrone

With such diversity of structure and bioactivity among the polyketide metabolites of *A. solani*, it is little wonder that so much research centres upon their isolation and appraisal. To date, however, most of these metabolites and their derivatives have been isolated from culture. Extraction and purification of culture isolates can be difficult and time consuming, especially if the desired compound is not present in appreciable quantities.

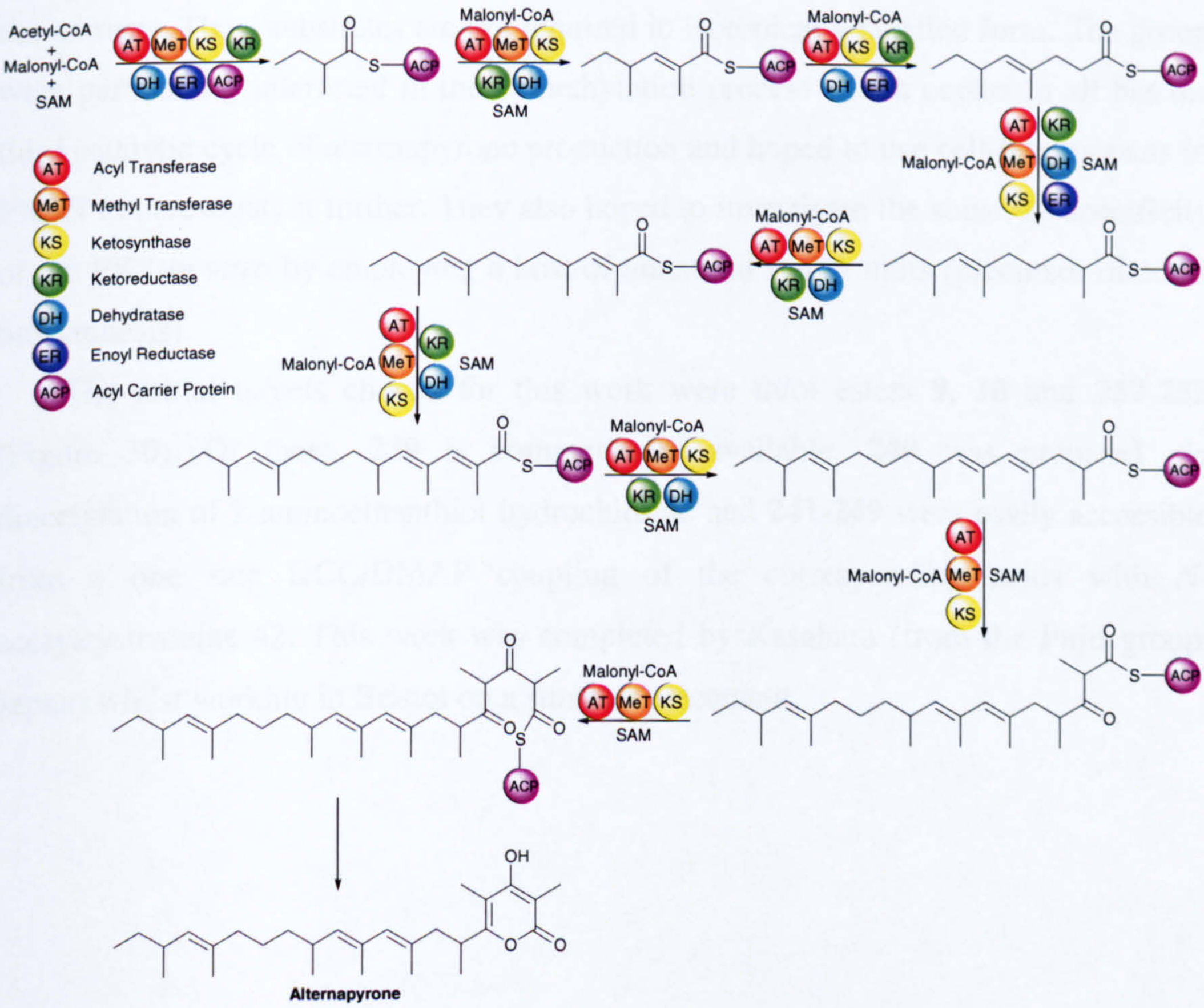
Recently, in an endeavour to explore polyketide biosynthesis in *A. solani*, Fujii *et al.* adopted a molecular genetic methodology which targeted PKS genes. The group designed a new degenerate primer pair, based on a conserved region of PKS DNA and used it to obtain a PKS gene fragment from *A. solani*.[157] This fragment was then used as a probe to screen for other PKS gene clusters within the genome, yielding a cluster of five genes, which they designated the *alt* gene cluster. Homology searching indicated that *alt1*, *alt2* and *alt3* encoded three cytochrome P450s, *alt4* encoded an FAD-dependent oxygenase/oxidase and *alt5* encoded an iterative type 1 polyketide synthase which the group denoted PKSN. Catalytic domain searching indicated that PKSN comprised of KS, AT, DH, MeT, KR, ER and ACP domains. Heterologous expression of PKSN in the fungal host *Aspergillus oryzae*, led to production of a novel polyketide metabolite, alternapyrone.

Elucidation of the structure of alternapyrone by NMR and mass spectrometry led to the structure shown in Scheme 90 though the geometries of the three double bonds

have not been unequivocally assigned and the configurations of the three stereogenic centres have yet to be assigned. Labelling experiments were carried out using [1,2-$^{13}C_2$]-acetate and [methyl-^{13}C]-methionine and while the backbone was found to be derived entirely from acetate units, the methyl substituents throughout the structure were derived from methionine. By analyzing this labelling pattern, the Fujii group proposed a biosynthetic pathway for alternapyrone (Scheme 91).



Scheme 90: Labelling pattern of alternapyrone from [1,2-$^{13}C_2$]-acetate and [methyl-^{13}C]-methionine.[156]



Scheme 91: Proposed biosynthetic pathway to alternapyrone.[156]

Interestingly, alternapyrone is thought to share the same biosynthetic pathway as the main chain of alternaric acid for the first four condensation cycles. However, the strain of *A. solani* used by Fujii does not produce alternaric acid and so alternapyrone is considered to be the correct product of PKSN and not a malformed or shunt product of the PKS for alternaric acid. It is also noteworthy that alternapyrone, or derivatives of alternapyrone, have not been detected in *A. solani* cultures thus far and so it seems highly likely that the alternapyrone produced by PKSN undergoes further modification by the P450s and FAD-dependent oxygenase/oxidase encoded by the rest of the *alt* gene cluster. This theory is currently under investigation by Fujii.

5.3 Synthesis of Putative Alternapyrone Precursors

In order to investigate the proposed biosynthetic pathway (Scheme 91) a partially purified cell-free extract was prepared by Fujii *et al.* with which to conduct feeding studies. The advantage of this system is that there is no background metabolite production and so any production observed must involve the substrates incubated with the extracts. Thus, substrates are not required in isotopically labelled form. The group were particularly interested in the *C*-methylation process which occurs in all but the third catalytic cycle of alternapyrone production and hoped to use cell-free extracts of PKSN to investigate it further. They also hoped to investigate the substrate specificity of the PKS *in vitro* by employing a host of unnatural starter units (precursor directed biosynthesis).

The initial targets chosen for this work were thiol esters **9**, **10** and **239-253** (Figure 30). Of these, **239** is commercially available, **240** was prepared *via* diacetylation of 2-aminoethanthiol hydrochloride and **241-249** were easily accessible from a one step DCC/DMAP coupling of the corresponding acids with *N*-acetylcysteamine **42**. This work was completed by Kasahara (from the Fujii group, Japan) whilst working in Bristol on a summer placement.

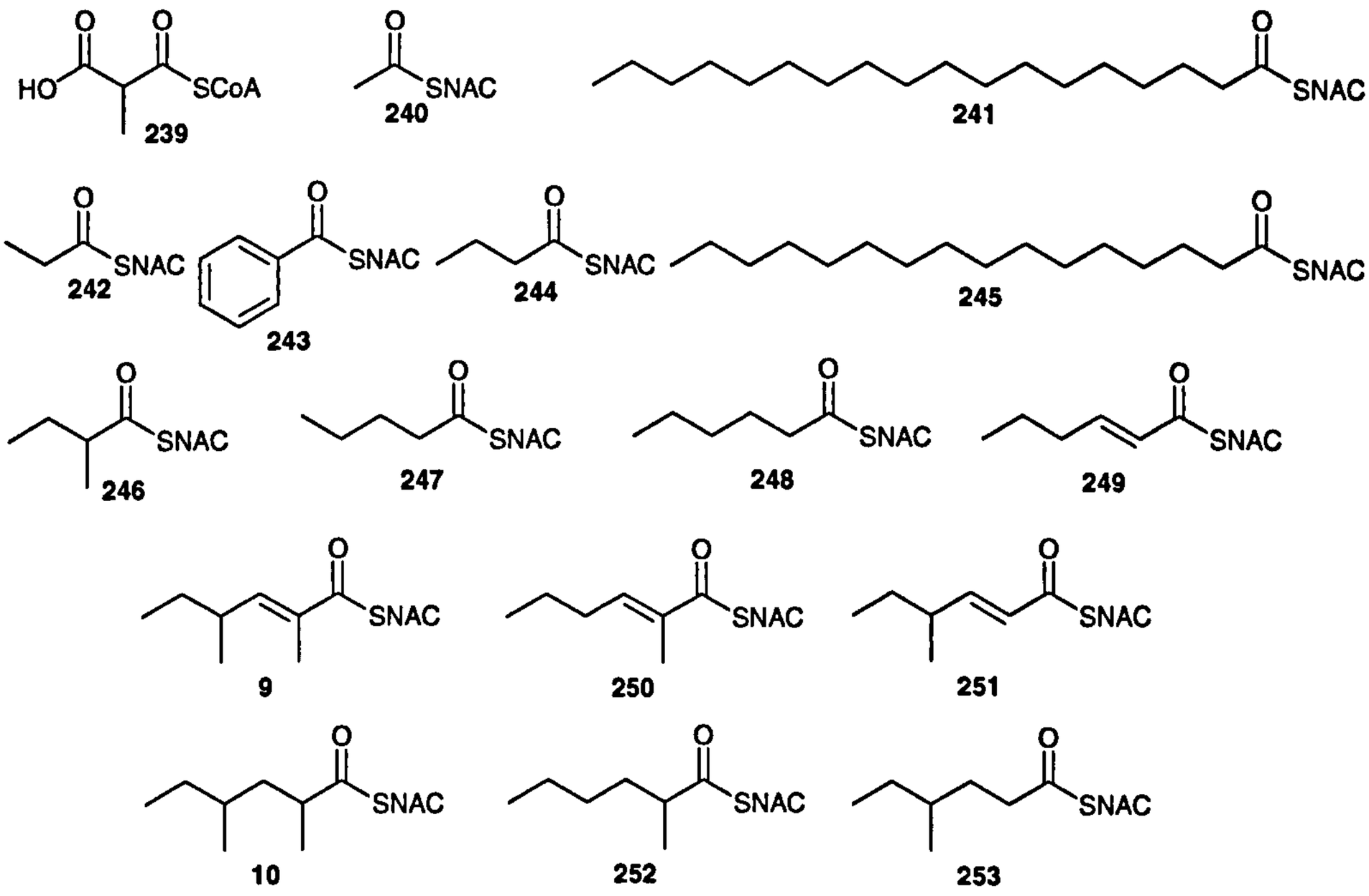
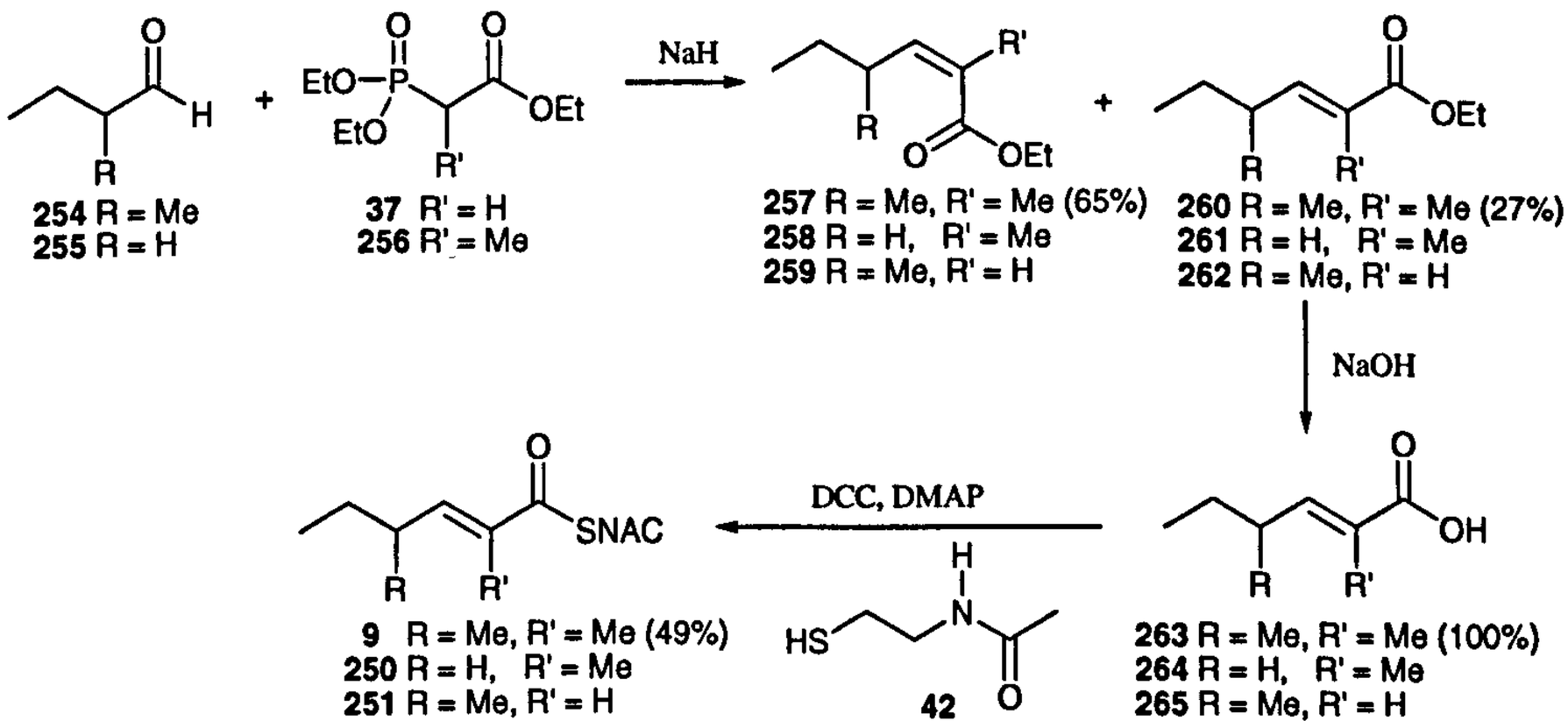


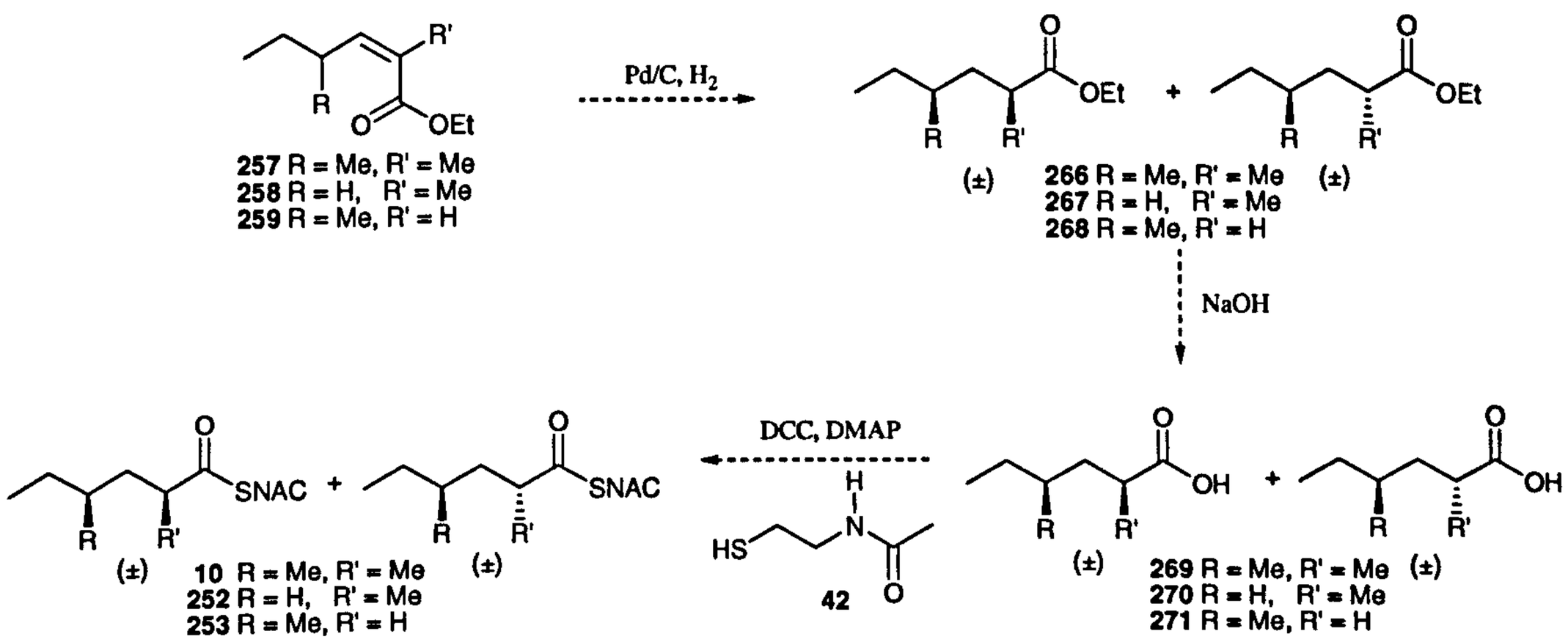
Figure 30: Substrates required to investigate alternapyrone biosynthesis.

The route chosen for the synthesis of thiol ester **9** is shown in Scheme 92. Starting from commercially available aldehyde **254** and triethyl 2-phosphonopropionate **256**, Horner-Wadsworth-Emmons reaction provided the unsaturated esters **257** and **260** in 65% and 27% yield respectively. Hydrolysis of **260** proceeded quantitively to produce the acid **263** which was then coupled with *N*-acetylcysteamine **42** to furnish the desired thiol ester **9** in 49% yield. This route was used by Kasahara to prepare thiol ester **250** from aldehyde **255** and triethyl 2-phosphonopropionate **256**, and thiol ester **251** from aldehyde **254** and triethyl phosphonoacetate **37**.



Scheme 92: Synthesis of thiol esters **9**, **250**[157] and **251**.[157]

It was proposed to synthesise thiol ester **10** from ethyl (*Z*)-2,4-dimethylhex-2-enoate **257** which was a by-product of the synthesis of thiol ester **9** (Scheme 93). Following the work of Morr *et al.*,[158] unsaturated ester **257** was dissolved in methanol and stirred under an atmosphere of hydrogen in the presence of palladium on activated charcoal. However, **257** was not soluble in methanol and after a day no reduction of the olefin was observed. The experiment was repeated using ethyl acetate as the solvent, but even after 5 days no saturated ester **266** was observed. Treatment of **257** with L-selectride appeared by ^{1}H NMR and T.L.C. to produce a small quantity of the desired ester but the reaction did not go to completion. Sodium borohydride in the presence of copper iodide also failed to reduce the olefin.

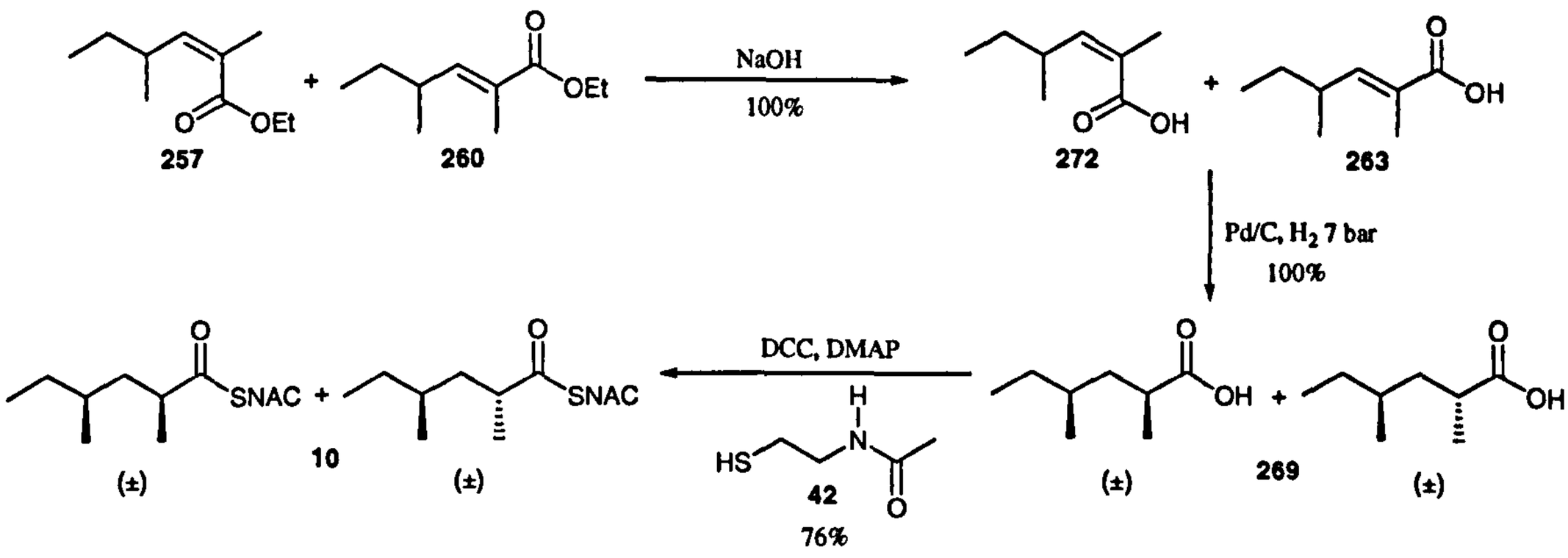


Scheme 93: Proposed route for the synthesis of thiol esters **10**, **252**[157] and **253**.[157]

Kasahara, on placement in Bristol, was simultaneously working on the synthesis of thiol esters **252** and **253**. He did not experience problems during the reduction step (and subsequently completed the synthesis of his targets as shown in Scheme 93). As his substrates, esters **258** and **259** differed from esters **257** and **260** only in the number of methyl substituents, it was proposed that the difficulty we were experiencing with our reduction was steric based. It was therefore decided to hydrolyse unsaturated esters **257** and **260** prior to reduction in the hope that the less bulky acids might be easier to hydrogenate.

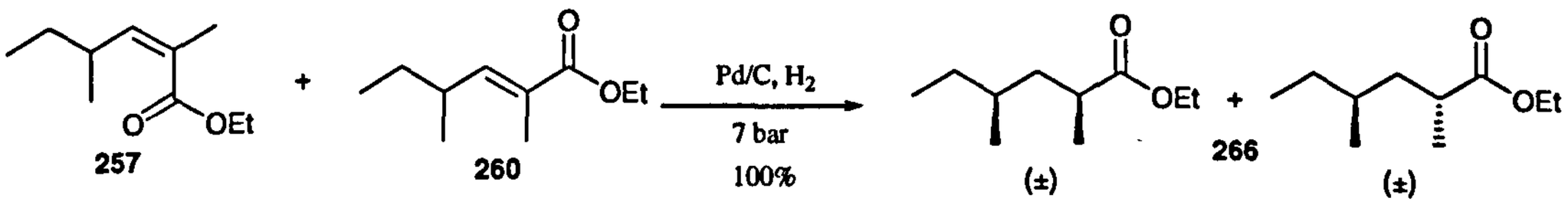
Hydrolysis of unsaturated esters **257** and **260** gave unsaturated acids **263** and **272** in quantitative yield (Scheme 94). This time hydrogenation in methanol reduced the *Z*-isomer to the saturated acid **269** but failed to convert the *E*-isomer. Encouraged by this result, the hydrogenation was repeated under 7 bar of pressure to furnish the

desired acid **269** as a 1:1 pair of diastereomers in quantitative yield. Coupling with *N*-acetylcysteamine in the presence of DCC and DMAP furnished the desired thiol ester **10** (also as a 1:1 pair of diastereomers) in 76% yield.



Scheme 94: Optimised route to thiol ester **10.**

It was interesting to speculate whether hydrogenation of esters **257** and **260** was possible under the same conditions employed for the acids **263** and **272**. A mixture of the esters **257** and **260** in methanol was stirred under an atmosphere of hydrogen (7 bars), in the presence of palladium on carbon for 3 days. Quantitative conversion to the desired saturated ester **266** as a 1:1 pair of diastereomers was observed (Scheme 95).

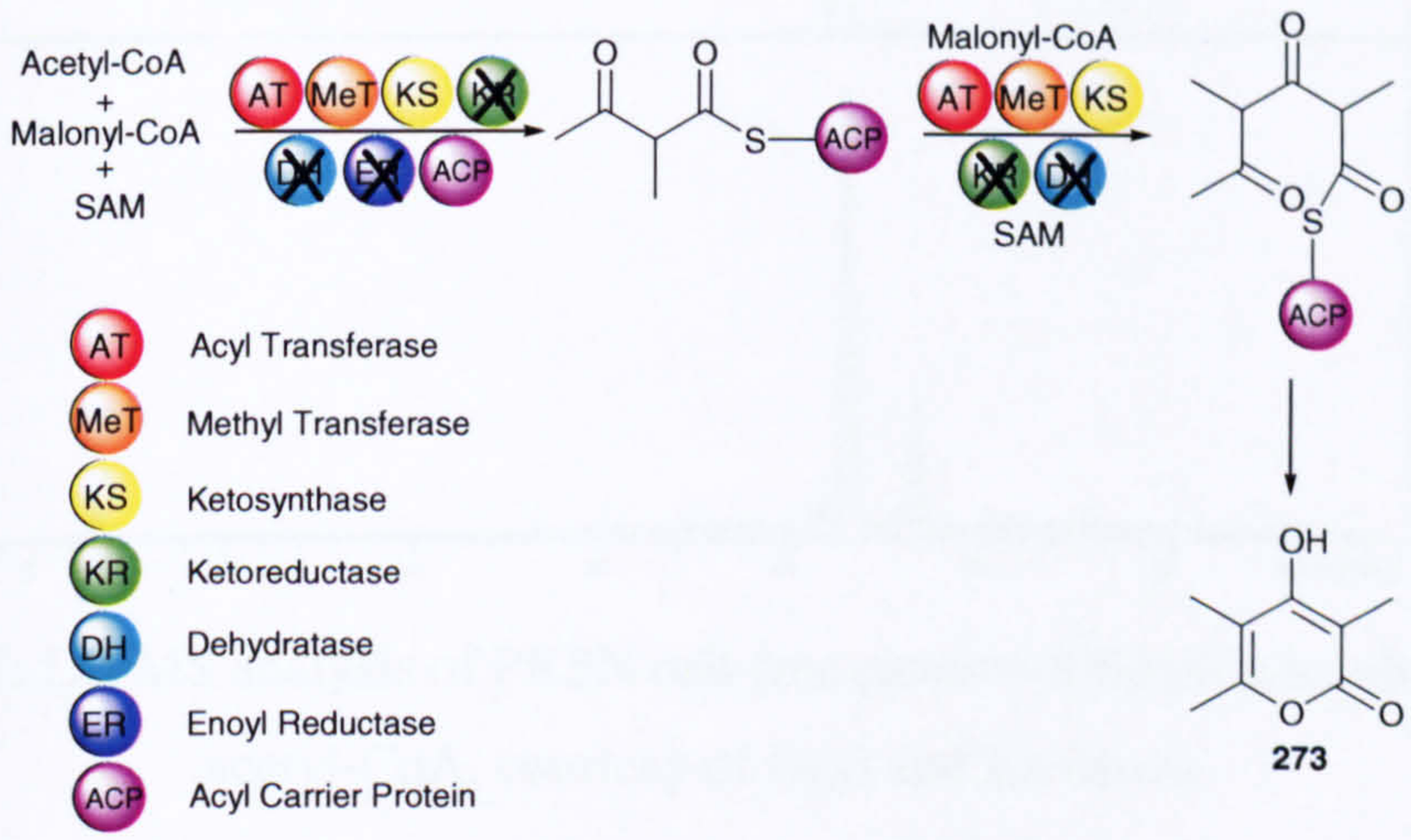


Scheme 95: Reduction of unsaturated esters **257** and **260** to saturated ester **266.**

Thiol esters **9, 10** and **239-253** were sent to Japan for feeding studies. Crude cell-free extracts of PKSN were prepared by grinding the fungal mycelia of the host fungus in liquid nitrogen and extracting with buffer solution. Unfortunately, feeding studies with the crude extracts were unsuccessful for all the substrates. Thus, the extracts were partially purified using a his-tag®.

Acetyl-CoA is the proposed starter unit for alternapyrone biosynthesis and therefore would be expected to prime the first round of chain extension. When all the correct substrates and co-factors are present (i.e. malonyl Co-A, SAM and NADPH), acetyl-CoA should produce alternapyrone when incubated with the PKSN extracts. In

the absence of the co-factor NADPH however, the reductive domains of the PKS are effectively rendered inactive and the chain might be expected to cyclise early to give the truncated product of the first and second rounds of condensation, dimethyltriacetic acid lactone (dimethylTAL) **273** (Scheme 96). When acetyl-CoA was incubated with the extracts solely in the presence of malonyl-CoA, SAM and buffer, a peak was observed by LC-MS which had a retention time of 26.5 minutes and a molecular mass of $MH^+ = 155$ (Figure 31). This was found to correspond to a synthetic standard of dimethylTAL **273**. Despite the lack of reduction in these cycles, *C*-methylation was not disrupted.



Scheme 96: Proposed biosynthesis of dimethylTAL **273** *in vitro*.

Thiol ester **9** is the proposed precursor of the third condensation cycle of alternapyrone biosynthesis. Thus, this compound might be expected to skip the first two condensation cycles and prime the third. As before, in the absence of NADPH, a truncated lactone would be expected and as the third condensation does not generally undergo *C*-methylation, the mono-methylated lactone **274** would be expected to form (Scheme 97). However, thiol ester **9** might also act as an unnatural starter unit priming the first condensation cycle in place of acetyl-CoA. In this case, the dimethylated lactone **276** would be the expected product. It is also possible that *C*-methylation would be disrupted by the unnatural substrates in either case, leading to the non-methylated lactone **275**.

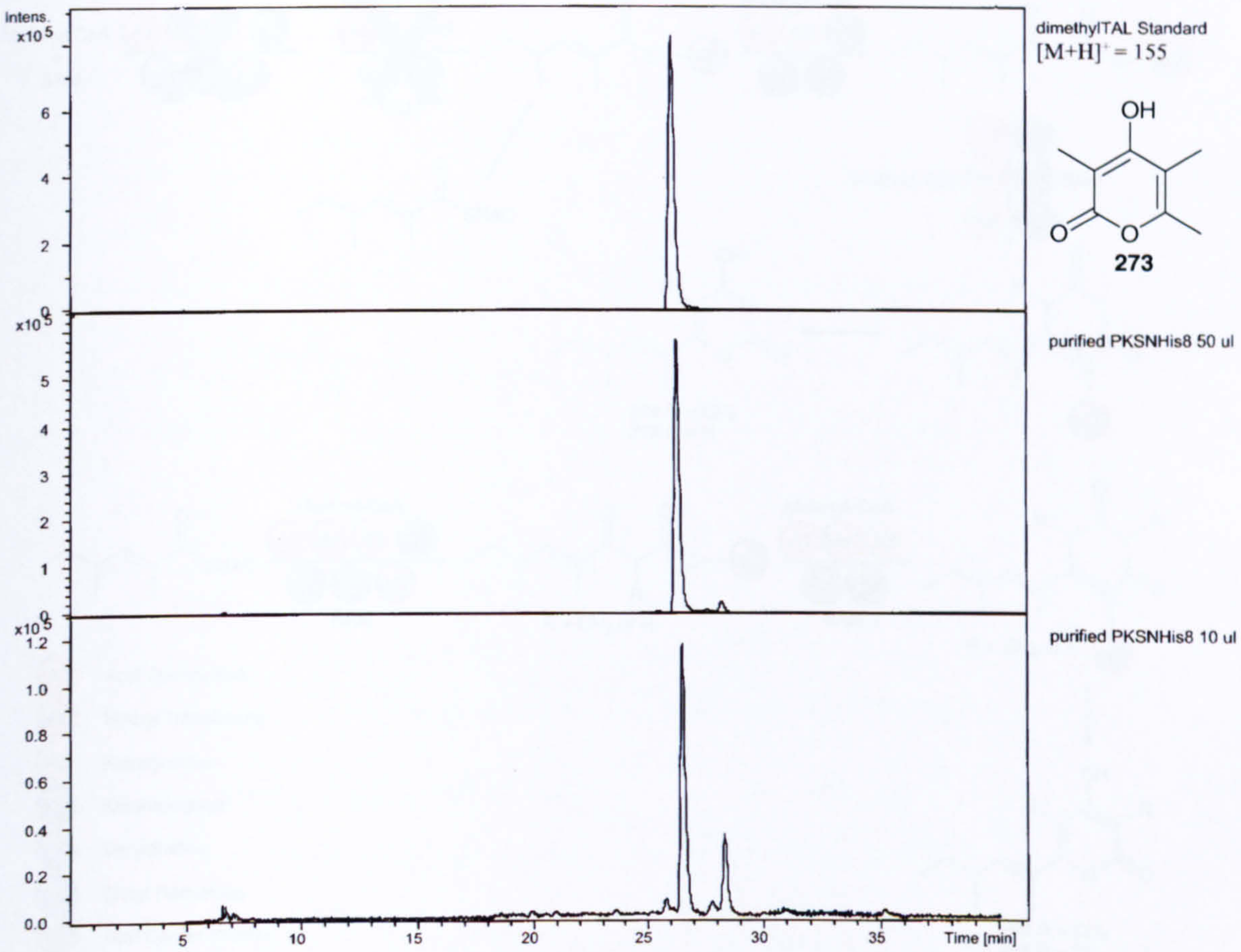
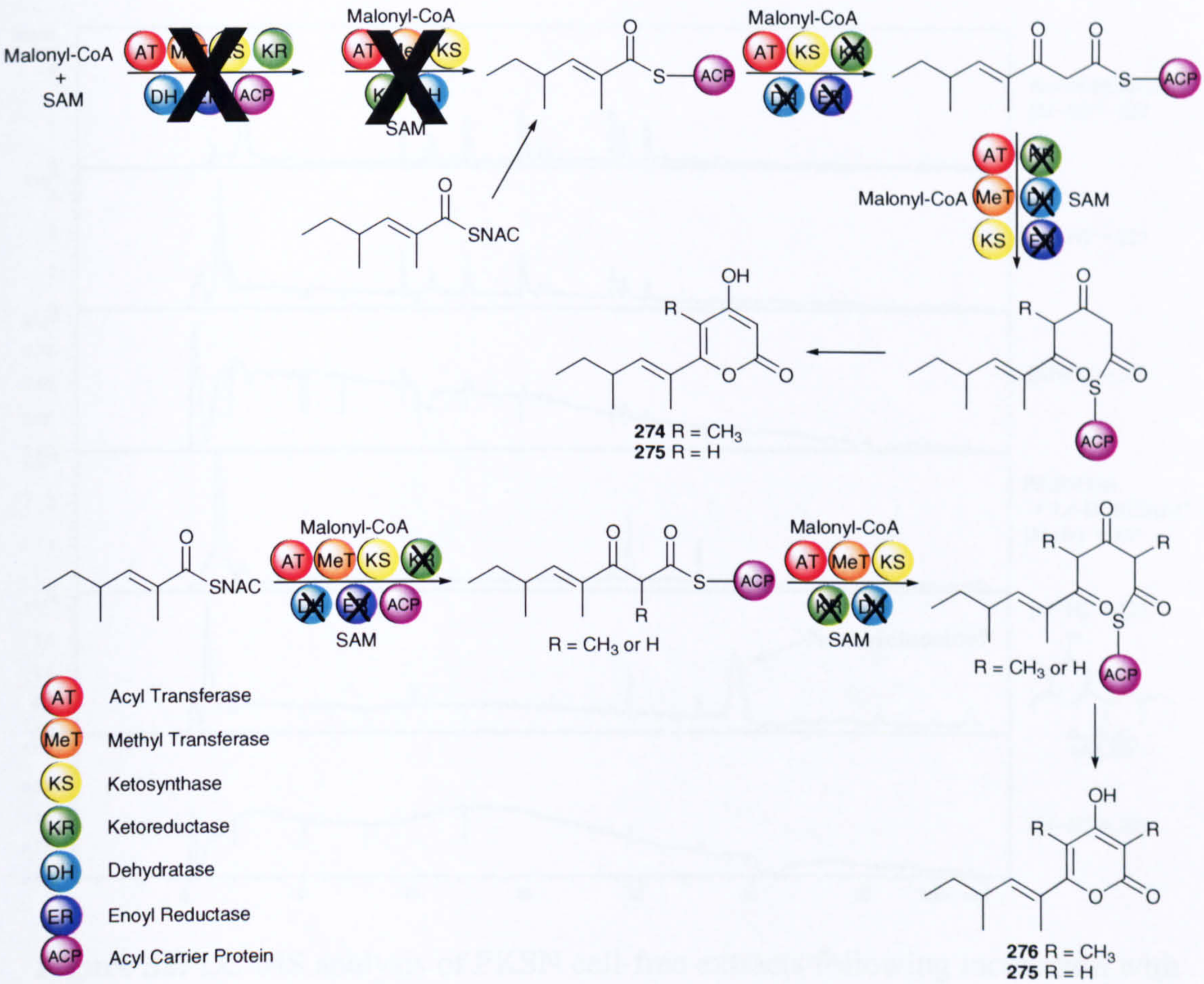


Figure 31: LC-MS analysis of PKSN cell-free extracts following incubation with acetyl-CoA, courtesy of Fujii and Kasahara.

When thiol ester **9** was incubated with the extracts in the presence of malonyl-CoA, SAM and buffer, production of a novel metabolite was observed by LC-MS (Figure 32). The new compound, which was not present in the control, had a mass of $MH^+ = 223$ and thus is proposed to be lactone **274**. No new peaks were observed at $MH^+ = 209$ (the expected mass of lactone **275**) or $MH^+ = 237$ (the expected mass of lactone **276**). This implies that thiol ester **9** primed the third condensation cycle and the *C*-methlyation pattern remained intact.

Unfortunately, due to time constraints on the part of Kasahara (of the Fujii group, Japan) and problems with protein degradation, thiol esters **10** and **239-253** were not fed to the partially purified PKSN extracts and thus no further results have been obtained for these feeding experiments to date.



Scheme 97: Two possible sites of incorporation of thiol ester **9** *in vitro*.

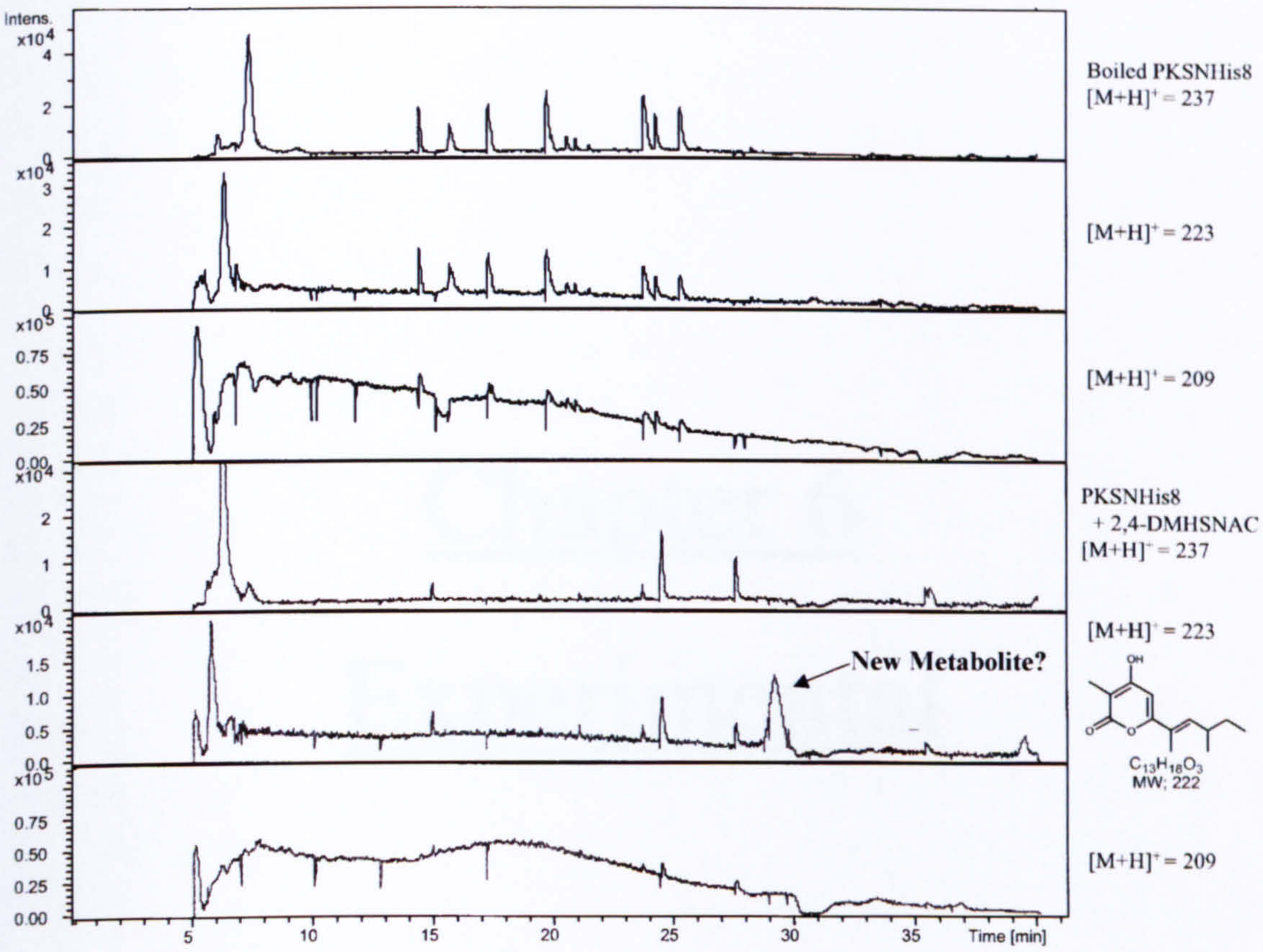


Figure 32: LC-MS analysis of PKSN cell-free extracts following incubation with thiol ester **9**, courtesy of Fujii and Kasahara.

5.4 Conclusions

A flexible route to putative alternapyrone precursors was devised and optimised. Thiol esters **9** and **10** were prepared *via* this route and fed to PKSN extracts (in the absence of NADPH) by Fujii and Kasahara. The feeding study with thiol ester **9** resulted in a new metabolite with a mass corresponding to lactone **274**. This indicated that thiol ester **9** had incorporated during the third condensation cycle and thus is likely to be the true substrate of that cycle. The *C*-methylation pattern was not disrupted by the unnatural substrate.

Thiol ester **10** did not incorporate during the initial feeding study and due to time constraints could not be repeated with the partially purified extracts.

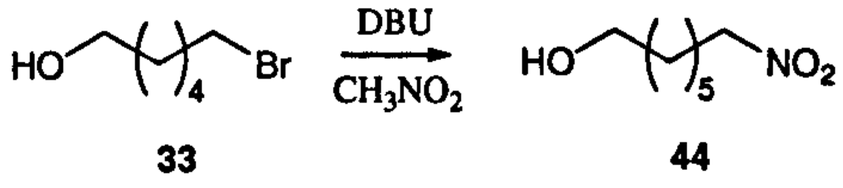
Chapter 6

Experimental

General Experimental Details

All commercially available compounds were used without further purification except where stated. All moisture or air sensitive reactions were carried out in flame-dried glassware under a positive pressure of nitrogen using standard syringe/septa techniques. Anhydrous solvents were obtained by passing through a modified Grubbs system of alumina columns, manufactured by Anhydrous Engineering. When stated, DMF was dried sequentially over three portions of 10% w/v 3Å molecular sieve beads for 24 h each, and then stored under nitrogen. Petroleum ether is of the 40-60 °C boiling point range. Routine monitoring of reactions was performed using precoated Merck-Keiselgel 60 F_{254} aluminium backed T.L.C. plates. The spots were visualised by UV_{254} light, or potassium permanganate. Flash column chromatography[159] was performed using silica gel (40-63 micron, obtained from Fluorochem Ltd.) as the adsorbent. Melting points were determined on an Electrothermal IA6301 melting point apparatus and are uncorrected. Optical rotations were recorded using a Bellingham and Stanley ADP220 polarimeter, irradiating with the sodium D line (λ = 589 nm), and $[\alpha]_D$ values are quoted in units 10^{-1} deg cm^2 g^{-1}. Infrared spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer in the solid or liquid state. ^1H and ^{13}C NMR spectra were recorded using either a Jeol Delta/GX 400 MHz or a Jeol Eclipse 400 MHz spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) and the coupling constants (J) are in Hertz (Hz) and rounded to the nearest half integer. Tetramethylsilane was used as the internal reference for proton and carbon chemical shifts. DEPT135, COSY, HMQC and HMBC NMR spectra were routinely used to definitively assign the signals of ^1H and ^{13}C NMR spectra. Electron impact (EI) and chemical ionisation (CI) mass spectra were recorded on a VG Analytical Autospec mass spectrometer. Electrospray (ESI) mass spectra were recorded on a Micromass LCT mass spectrometer or a VG Quattro mass spectrometer. Methane was the ionisation gas used for chemical ionisation. X-ray crystallography data was collected by the EPSRC National Crystallography Service and interpreted by Dr. J.P.H. Charmant. Elemental analysis was carried out using a Eurovector EA3000 elemental analyser or a Carolerba NA2500 elemental analyser. Unless stated, data for all known compounds are in agreement with published data.

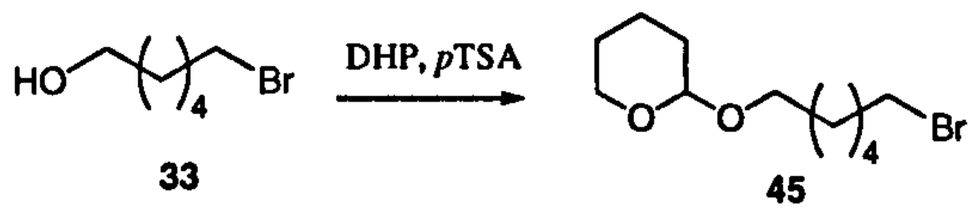
7-Nitroheptan-1-ol 44[160]



33 44

1,8-Diazabicyclo[5.4.0]undec-7-ene (0.42 g, 2.76 mmol) was added to nitromethane (83 mL) at 0 °C and stirred for 10 minutes. 6-Bromo-1-hexanol **33** (0.50 g, 2.76 mmol) was added and the solution was heated to reflux for 3 days. The solvent was then removed *in vacuo*. Water (110 mL) and diethyl ether (80 mL) were added and the organic layer was separated. The aqueous layer was extracted with diethyl ether (2 × 80 mL) and the combined organic layers were washed with hydrochloric acid (1.0 M, 80 mL) and brine (80 mL). The solution was dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was then purified by flash chromatography (SiO_2, 5% methanol/ethyl acetate) to furnish alcohol **44** (0.18 g, 40%) as a yellow oil. υ_{max}(neat)/cm^{-1} 3350 (OH), 2930, 2840, 1550 (NO_2), 1380 (NO_2); δ_H(400 MHz) 1.39-1.63 (8H, m, 4 × CH_2), 1.97-2.06 (2H, m, CH_2), 3.65 (2H, t, *J* 6.5, C*H*$_2$OH), 4.39 (2H, t, *J* 7.0, C*H*$_2$$NO_2$); *m/z* (EI) 161 ($M^+$, 13%), 145 (19), 105 (100), 91 (40), 83 (12), 77 (41), 69 (15) and 57 (45).

6-(1′-Tetrahydropyran-2-yl)oxy-1-bromohexane 45[161]

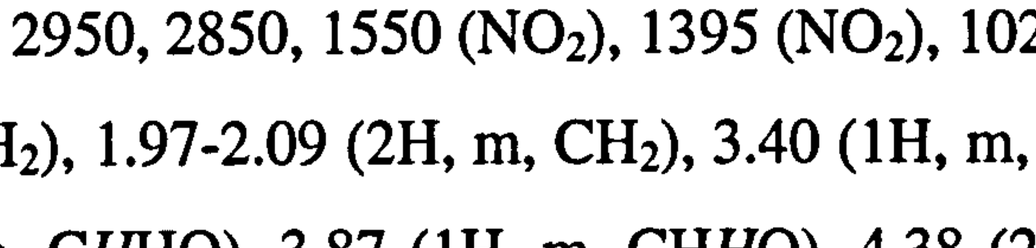


33 45

To a stirred solution of 6-bromo-1-hexanol **33** (0.50 g, 2.76 mmol) in diethyl ether (2.5 mL) was added dihydropyran (0.23 g, 2.76 mmol) in diethyl ether (1 mL) and *p*-toluenesulfonic acid (0.05 g, 0.27 mmol). The mixture was stirred at room temperature for 24 hours and then quenched with water (12 mL) prior to extraction with ethyl acetate (3 × 15 mL). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to afford tetrahydropyran **45** (0.71 g, 97%) as a colourless oil. This was used without further purification. δ_H(400 MHz) 1.35-1.92 (14H, m, 7 × CH_2), 3.39 (1H, dt, *J* 9.5, 7.0, C*H*HO), 3.41 (2H, t, *J* 7.0, CH_2Br), 3.52 (1H, m, CH*H*O), 3.74 (1H, dt, *J* 10.0, 7.0, C*H*HO), 3.86 (1H, m, C*H*HO), 4.57 (1H, m, OCHO); δ_C(100 MHz) 19.7 (CH_2), 25.5 (CH_2), 25.5 (CH_2), 28.0 (CH_2), 29.6 (CH_2), 30.8 (CH_2), 32.8 (CH_2), 33.8 (CH_2), 62.4 and 67.4 (2 × CH_2O), 98.9 (OCHO), *m/z* (CI) 267/265 (M^+, 25/27%), 169 (22), 165/163 (7/7) and 85 (100).

7-(1′-Tetrahydropyran-2-yl)-oxy-1-nitroheptane 46[162]

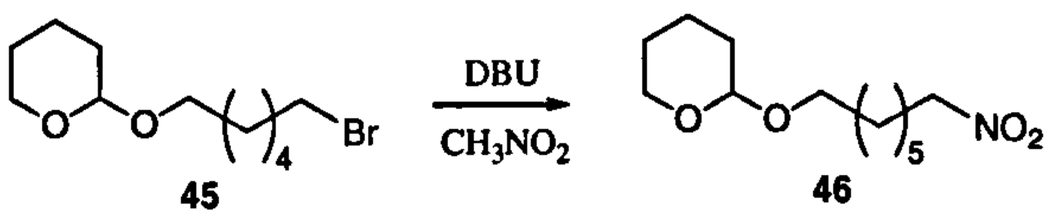
(a) *via* protection of 7-nitroheptan-1-ol 44



44 46

To a stirred solution of alcohol **44** (0.82 g, 5.09 mmol) in diethyl ether (5 mL) was added dihydropyran (0.43 g, 5.09 mmol) in diethyl ether (2 mL) and a catalytic amount of *p*-toluenesulfonic acid (0.1 g, 0.53 mmol). The mixture was stirred at room temperature for 27 hours and was then quenched with water (30 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to afford tetrahydropyran **46** (0.88 g, 70%) as an orange oil. This was used without further purification. υ_{max}(neat)/cm^{-1} 3400, 2950, 2850, 1550 (NO_2), 1395 (NO_2), 1020; δ_H(400 MHz) 1.35-1.93 (14H, m, 7 × CH_2), 1.97-2.09 (2H, m, CH_2), 3.40 (1H, m, C*H*HO), 3.52 (1H, m, CH*H*O), 3.74 (1H, m, C*H*HO), 3.87 (1H, m, CH*H*O), 4.38 (2H, t, *J* 7.0, CH_2NO_2), 4.58 (1H, m, OCHO); δ_C(100 MHz) 19.7 (CH_2), 25.5 (CH_2), 25.7 (CH_2), 26.1 (CH_2), 27.4 (CH_2), 29.4 (CH_2), 30.7 (CH_2), 30.8 (CH_2), 62.5 (CH_2O), 63.0 (CH_2O), 75.7 (CH_2NO_2), 99.0 (OCHO).

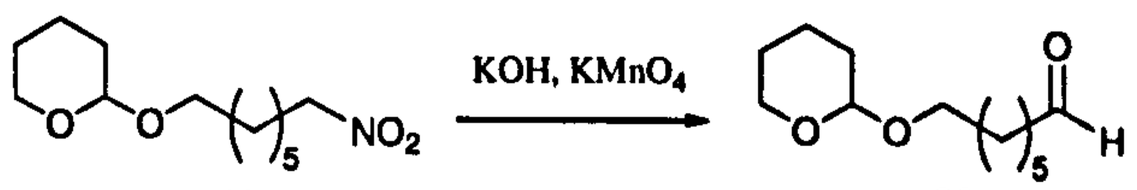
(b) *via* nitration of 6-(1′-tetrahydropyran-2-yl)oxy-1-bromohexane 45



45 46

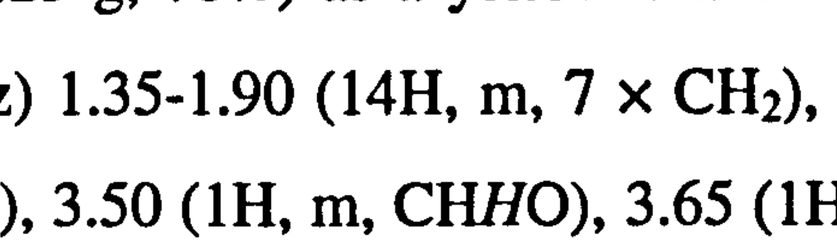
1,8-Diazabicyclo[5.4.0]undec-7-ene (1.46 g, 9.58 mmol) was added to nitromethane (20 mL) at 0 °C and was stirred for 10 minutes. Tetrahydropyran **45** (2.54 g, 9.58 mmol) was added and the solution was heated to reflux for 25 hours. The solvent was then removed *in vacuo*. Water (200 mL) and ethyl acetate (200 mL) were added and the organic layer was separated. The aqueous layer was extracted with diethyl ether (2 × 200 mL) and the combined organic layers were washed with hydrochloric acid (1.0 M, 100 mL) and brine (100 mL). The solution was then dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield the crude product. Attempts to separate the desired product from the mixture of products formed were unsuccessful. However, spectral analysis confirmed the presence of tetrahydropyran **46**. Spectral data as quoted above.

7-(1′-Tetrahydropyran-2-yl)oxy-heptan-1-al 36[162]



Tetrahydropyran **46** (1.93 g, 7.88 mmol) in methanol (55 mL) was cooled to 0 °C under an atmosphere of nitrogen. A solution of potassium hydroxide (0.66 g, 11.82 mmol) in methanol (80 mL) was added dropwise over 35 minutes and the solution was stirred for an additional 15 minutes. A solution of potassium permanganate (1.25 g, 7.88 mmol) and magnesium sulfate (1.42 g, 11.82 mmol) in water (120 mL) was slowly added, maintaining the reaction temperature at 0 °C. Upon complete addition, the reaction mixture was stirred for a further 30 minutes and then filtered through a layer of florisil®. The filtrate was evaporated to remove most of the methanol and the resulting aqueous phase was extracted with ethyl acetate (3 × 40 mL). The combined organic phases were dried over magnesium sulfate, filtered and concentrated *in vacuo* to afford aldehyde **36** (1.23 g, 78%) as a yellow oil. This was used without further purification. δ_H(400 MHz) 1.35-1.90 (14H, m, 7 × CH_2), 2.45 (2H, td, *J* 7.0, 2.0, 2-H_2), 3.40 (1H, m, C*H*HO), 3.50 (1H, m, CH*H*O), 3.65 (1H, m, C*H*HO), 3.74 (1H, m, CH*H*O), 4.58 (1H, m, OCHO), 9.77 (1H, t, *J* 2.0, CHO); δ_C(100 MHz) 19.8 (CH_2), 25.5 (CH_2), 26.0 (CH_2), 26.2 (CH_2), 29.7 (CH_2), 30.8 (CH_2), 32.7 (CH_2), 43.9 (C-2), 63.0 (CH_2O), 67.6 (CH_2O), 98.9 (OCHO), 162.8 (C-1).

Triethyl Phosphonoacetate 37[163]



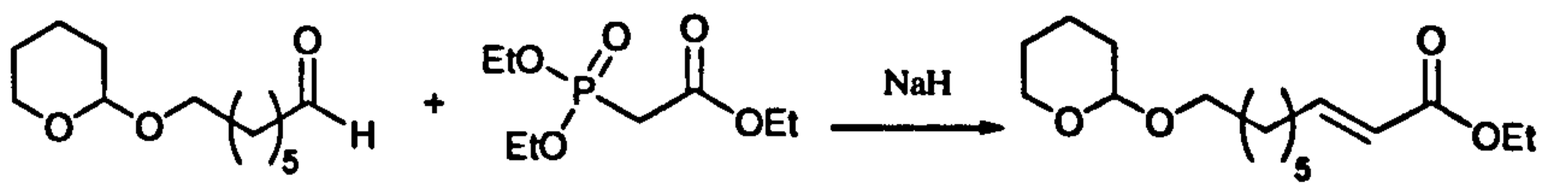
Triethylphosphite (3.32 g, 20.00 mmol) was added to a solution of ethyl bromoacetate **49** (2.80 g, 16.76 mmol) in dry toluene (40 mL) and the reaction mixture was heated to reflux for 48 hours. The solvent was then removed *in vacuo* to yield triethyl phosphonoacetate **37** (3.65 g, 97%) as a colourless oil. This was used without further purification. δ_H(400 MHz) 1.29 (3H, t, *J* 7.0, C*H*$_3$CH$_2$OCO), 1.35 (6H, t, *J* 7.0, 2 × C*H*$_3$CH$_2$OP), 2.97 (2H, d, *J* 21.5, CH_2P), 4.08-4.23 (6H, m, 3 × CH_3CH_2O); δ_C(100 MHz) 14.1 (*C*H$_3$CH$_2$OCO), 16.2 and 16.4 (each d, *J* 6.0, 2 × *C*H$_3$CH$_2$OP), 34.5 (d, *J* 134.5, *C*H$_2$P), 61.6 (CH$_3$*C*H$_2$OCO), 62.7 and 63.7 (each d, *J* 6.0, 2 × CH$_3$*C*H$_2$OP),

165.8 (d, *J* 6.0, CO); *m/z* (EI) 224 (M^+, 17%), 197 (79), 179 (90), 169 (48), 151 (80), 123 (100), 109 (60), 88 (42) and 81 (62).

[1,2-$^{13}C_2$]-Triethyl Phosphonoacetate 51[164]

The above procedure was repeated using ethyl [1,2-$^{13}C_2$]-bromoacetate **50** (0.25 g, 1.50 mmol) to yield [1,2-$^{13}C_2$]-triethyl phosphonoacetate **51** (0.33 g, 100%) as a colourless oil. δ_H(400 MHz) 1.29 (3H, t, *J* 7.0, C*H*$_3$CH$_2$OCO), 1.35 (6H, t, *J* 7.0, 2 × C*H*$_3$CH$_2$OP), 2.96 (2H, ddd, *J* 130.0, 21.5, 8.0, CH$_2$P), 4.08-4.24 (6H, m, 3 × CH$_3$C*H*$_2$O); δ_C(100 MHz) signals assigned to C-1 (166.2 ppm) and C-2 (34.5 ppm) show an enhancement of >95% (based on ^{1}H NMR and MS data) 14.5 (d, *J* 2.0, *C*H$_3$CH$_2$OCO), 16.5 and 16.7 (each d, *J* 6.0, *C*H$_3$CH$_2$OP), 34.5 (dd, *J* 134.0, 58.5, C-2), 61.9 (d, *J* 2.0, CH$_3$*C*H$_2$OCO), 63.0 and 64.0 (d, *J* 6.0, 2 × CH$_3$*C*H$_2$OP), 166.2 (dd, *J* 58.5, 6.0, C-1); *m/z* (CI) 227 (MH^+, 100), 199 (26), 181 (28), 153 (7), 86 (9) and 84 (15).

Ethyl (2*E*)-9-(1′-tetrahydropyran-2-yl)oxynon-2-enoate 38[165]

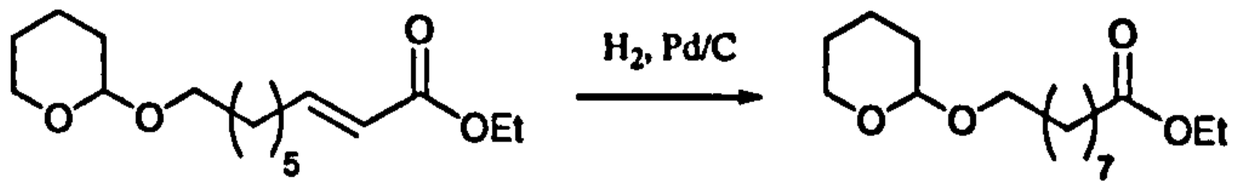


36 **37** **38**

Sodium hydride (60% dispersion in oil prewashed in hexane, 0.11 g, 4.69 mmol) was stirred in THF (80 mL) under an atmosphere of nitrogen. Triethyl phosphonoacetate **37** (0.85 g, 3.80 mmol) in THF (10 mL) was added dropwise at 0 °C and stirred for 30 minutes. Aldehyde **36** (0.72 g, 3.36 mmol) in THF (10 mL) was added dropwise at 0 °C and the resulting solution was stirred overnight at room temperature. The reaction mixture was quenched with water (200 mL) and was extracted with ethyl acetate (3 × 200 mL). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo*. Purification by flash column chromatography (SiO_2, 10% ethyl acetate/petroleum ether 40-60 °C) afforded unsaturated ester **38** (0.28 g, 30%) as a pale yellow oil. δ_H(400 MHz) 1.29 (3H, t, *J* 7.0, C*H*$_3$CH$_2$O), 1.35-1.90 (14H, m, 7 × CH$_2$), 2.22 (2H, app. qd, *J* 7.0, 1.5, 4-H$_2$), 3.38 (1H, m, C*H*HO), 3.50 (1H, m, CH*H*O), 3.73 (1H, dt, *J* 9.5, 7.0, OC*H*H), 3.87 (1H, m, OCH*H*), 4.18 (2H, q, *J* 7.0, CH$_3$C*H*$_2$O), 4.57 (1H, m, OCHO), 5.82 (1H, dt, *J* 15.5, 1.5, 2-H), 6.96 (1H, dt, *J* 15.5, 7.0, 3-H); δ_C(100 MHz) 14.3 (*C*H$_3$CH$_2$O), 19.7 (CH$_2$), 25.5 (CH$_2$), 25.8 (CH$_2$), 26.1 (CH$_2$), 27.9 (CH$_2$), 29.7 (CH$_2$), 30.8 (CH$_2$), 32.1 (CH$_2$), 60.1 (CH$_3$*C*H$_2$O), 62.4

(CH_2O), 67.6 (CH_2O), 98.8 (OCHO), 121.4 (C-2), 149.2 (C-3), 166.8 (C-1); *m/z* (CI) 285 (MH^+, 20%), 271 (71), 255 (19) 241 (28), 225 (40), 216 (19), 203 (19), 187 (26) and 85 (100).

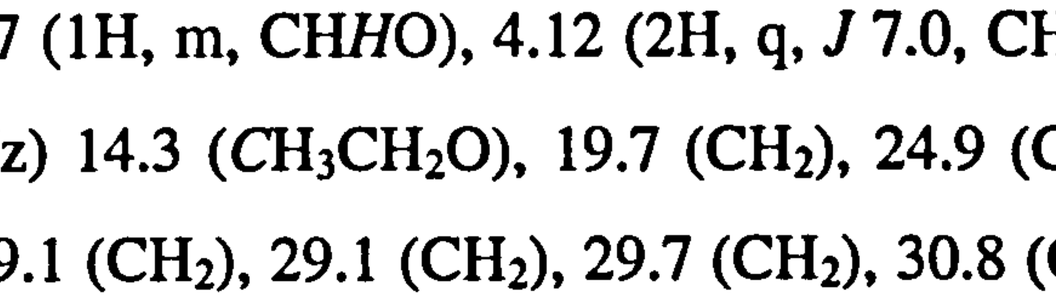
Ethyl 9-(1′-tetrahydropyran-2-yl)oxynonanoate 39[67]



38 **39**

To a solution of unsaturated ester **38** (0.10 g, 0.46 mmol) in methanol (5 mL) was added palladium on activated charcoal (≈40 mg) and the reaction mixture was stirred at room temperature under an atmosphere of hydrogen for 4 hours. The solution was then filtered in order to remove the catalyst and the solvent was removed *in vacuo* to afford saturated ester **39** as a pale yellow oil (0.10 g, 100%). This was used without further purification. δ_H(400 MHz) 1.25 (3H, t, *J* 7.0, C*H*$_3$CH$_2$O), 1.30-1.90 (18H, m, 9 × CH_2), 2.29 (2H, t, *J* 7.5, 2-H_2), 3.38 (1H, m, C*H*HO), 3.50 (1H, m, CH*H*O), 3.72 (1H, m, C*H*HO), 3.87 (1H, m, CH*H*O), 4.12 (2H, q, *J* 7.0, CH$_3$C*H*$_2$O), 4.58 (1H, m, OCHO); δ_C(100 MHz) 14.3 (*C*H$_3$CH$_2$O), 19.7 (CH_2), 24.9 (CH_2), 25.5 (CH_2), 26.1 (CH_2), 26.2 (CH_2), 29.1 (CH_2), 29.1 (CH_2), 29.7 (CH_2), 30.8 (CH_2), 34.3 (CH_2), 60.1 (CH$_3$*C*H$_2$O), 62.3 (CH_2O), 67.5 (CH_2O), 98.8 (OCHO), 173.8 (C-1); *m/z* (CI) 286 (M^+, 1%), 271 (21), 227 (11), 125 (15), 119 (50), 111 (17), 85 (100), 83 (35) and 55 (21).

7-Hydroxyheptanal 48[166,167]

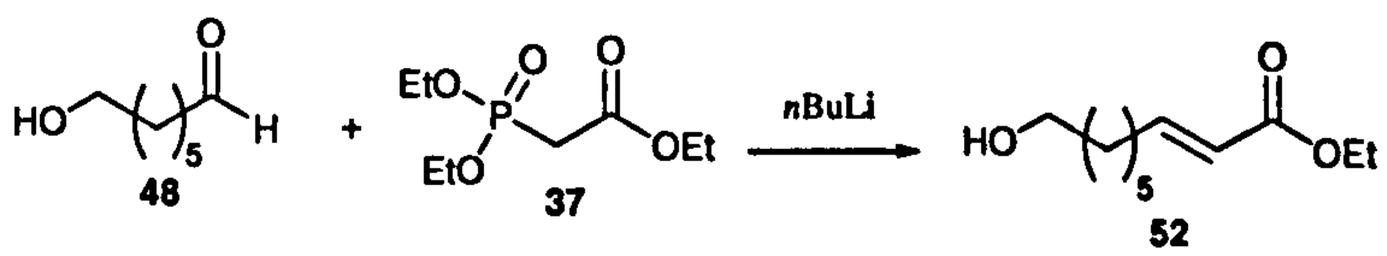


47 **48**

Aleuritic acid **47** (6.2 g, 20.00 mmol) and sodium periodate (6.40 g, 30.00 mmol) were dissolved in a solution of THF (150 mL) and water (40 mL). A white precipitate began to form almost immediately. The solution was stirred overnight at room temperature. Water (50 mL) was then added and the solution was extracted with ethyl acetate (3 × 150 mL). The combined organic extracts were subsequently washed with saturated aqueous sodium hydrogen carbonate solution (3 × 150 mL) and water (100 mL) and then dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield a yellow oil, which solidified upon cooling. Recrystallisation from ethyl acetate

and petroleum ether 40-60 °C furnished aldehyde **48** (2.02 g, 76%) as a white crystalline solid. m.p. 68-70 °C (from ethyl acetate/petroleum ether 40-60 °C), lit.[167] 65-67 °C, δ_H(400 MHz) 1.30-1.42 (4H, m, 2 × CH_2), 1.54-1.70 (4H, m, 2 × CH_2), 1.85 (1H, br s, OH), 2.45 (2H, td, *J* 7.0, 2.0, 2-H_2), 3.64 (2H, t, *J* 7.0, 7-H_2), 9.77 (1H, t, *J* 2.0, 1-H); δ_C(100 MHz) 22.0 (CH_2), 25.5 (CH_2), 28.9 (CH_2), 32.5 (CH_2), 43.8 (C-2), 62.8 (C-7), 202.7 (C-1); *m/z* (CI) 131 (MH^+, 2%), 113 (21), 95 (100), 69 (23) and 57 (9).

Ethyl (*E*)-9-Hydroxynon-2-enoate 52[165]



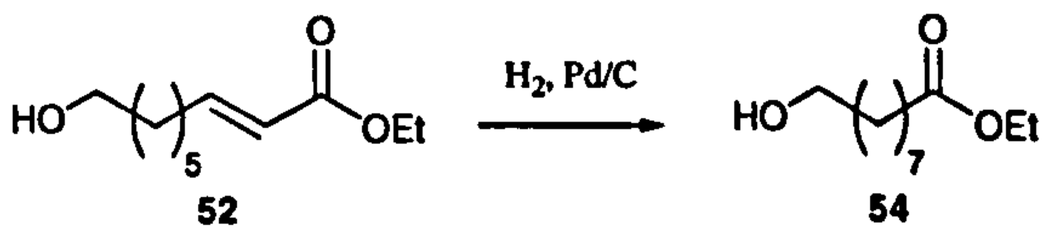
Triethyl phosphonoacetate **37** (1.8 mL, 8.96 mmol) in dry THF (48 mL) was cooled to 0 °C under an atmosphere of nitrogen. *n*-Butyllithium (2.5 M in hexanes, 3.6 mL, 8.96 mmol) was added and the reaction was stirred for 1 hour. Aldehyde **48** (1.74 g, 13.44 mmol) in dry THF (6 mL) was then added dropwise very slowly (in order to prevent a gum like precipitate which had previously been observed). The resulting solution was stirred for 18 hours at room temperature. The reaction was quenched with water (50 mL) and was extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo*. Purification by flash column chromatography (SiO_2, 20% ethyl acetate/petroleum ether 40-60 °C) afforded unsaturated ester **52** (1.47 g, 82%) as a pale yellow oil. δ_H(400 MHz) 1.29 (3H, t, *J* 7.0, CH_3), 1.34-1.61 (8H, m, 4 × CH_2), 2.19 (2H, app. qd, *J* 7.5, 1.5, 4-H_2), 3.64 (2H, t, *J* 6.5, 9-H_2), 4.19 (2H, q, *J* 7.0, CH_3CH_2O), 5.82 (1H, dt, *J* 15.5, 1.5, 2-H), 6.96 (1H, dt, *J* 15.5, 7.5, 3-H); δ_C(100 MHz) 14.3 (CH_3), 25.6 (CH_2), 28.0 (CH_2), 28.9 (CH_2), 32.1 (CH_2), 32.7 (CH_2), 60.2 (CH_3CH_2O), 63.0 (C-9), 121.4 (C-2), 149.2 (C-3), 166.8 (C-1); *m/z* (CI) 201 (MH^+, 12%), 197 (15), 155 (100), 137 (27), 127 (13), 109 (75), 95 (20) and 84 (17).

Ethyl [1,2-$^{13}C_2$]-(*E*)-9-Hydroxynon-2-enoate 53

The above reaction was repeated using [1,2-$^{13}C_2$]-triethyl phosphonoacetate **51** (0.25 g, 1.12 mmol) to give unsaturated ester **53** (0.20 g, 91%). δ_H(400 MHz) 1.29 (3H, t, *J* 7.0, CH_3), 1.34-1.40 (4H, m, 2 × CH_2), 1.43-1.52 (2H, m, CH_2), 1.52-1.61 (2H, m,

CH_2), 2.20 (2H, app. quint., *J* 7.0, 4-H_2), 3.61-3.66 (2H, m, 9-H_2), 4.14-4.22 (2H, m, CH_3CH_2O), 5.82 (1H, ddm, *J* 162.0, 15.5, 2-H), 6.96 (1H, m, 3-H); δ_C(100 MHz) signals assigned to C-1 (166.8 ppm) and C-2 (121.4 ppm) appear as doublets (*J* 75.0) and show an enhancement of >95% (based on ^{1}H NMR and MS data); *m/z* (CI) 203 (MH^+, 94%), 185 (26), 157 (53), 139 (20), 110 (72), 85 (68) and 83 (100).

Ethyl 9-Hydroxynonanoate 54[168]

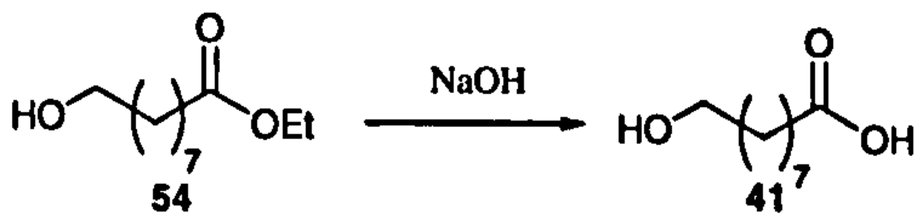


To a solution of unsaturated ester **52** (0.17 g, 0.60 mmol) in methanol (5 mL) was added palladium on activated charcoal (≈30 mg) and the reaction was stirred at room temperature under an atmosphere of hydrogen for 22 hours. The solution was then filtered in order to remove the catalyst and the solvent was removed *in vacuo* to afford saturated ester **54** as a colourless oil (0.17 g, 100%). This was used without further purification. δ_H(400 MHz) 1.26 (3H, t, *J* 7.0, CH_3), 1.29-1.42 (8H, m, 4 × CH_2), 1.55-1.68 (4H, m, 2 × CH_2), 2.29 (2H, t, *J* 7.5, 2-H_2), 3.64 (2H, t, *J* 6.5, 9-H_2), 4.13 (2H, q, *J* 7.0, CH_3CH_2O); δ_C(100 MHz) 14.29 (CH_3), 25.0 (CH_2), 25.7 (CH_2), 29.1 (CH_2), 29.2 (2 coincident signals, 2 × CH_2), 32.8 (CH_2), 34.4 (C-2), 60.2 (CH_3CH_2O), 63.1 (C-9), 173.9 (C-1); *m/z* (CI) 203 (MH^+, 49%), 185 (39), 157 (45), 139 (100), 121 (21), 115 (15), 97 (16) and 84 (30).

Ethyl [1,2-$^{13}C_2$]-9-Hydroxynonanoate 55

The above reaction was repeated using ester **53** (0.16 g, 0.79 mmol) to give ester **55** (0.16 g, 100%). δ_H(400 MHz) 1.25 (3H, t, *J* 7.0, CH_3), 1.29-1.39 (8H, m, 4 × CH_2), 1.51-1.63 (4H, m, 2 × CH_2), 2.29 (2H, dm, *J* 127.5, 2-H_2), 3.60-3.67 (2H, m, 9-H_2), 4.08-4.17 (2H, m, CH_3CH_2O); δ_C(100 MHz) signals assigned to C-1 (173.9 ppm) and C-2 (34.4 ppm) appear as doublets (*J* 57.0) and show an enhancement of >95% (based on ^{1}H NMR and MS data); *m/z* (CI) 205 (MH^+, 12 %), 187 (18), 159 (33), 141 (100), 123 (15) and 83 (23).

9-Hydroxynonanoic Acid 41[67]



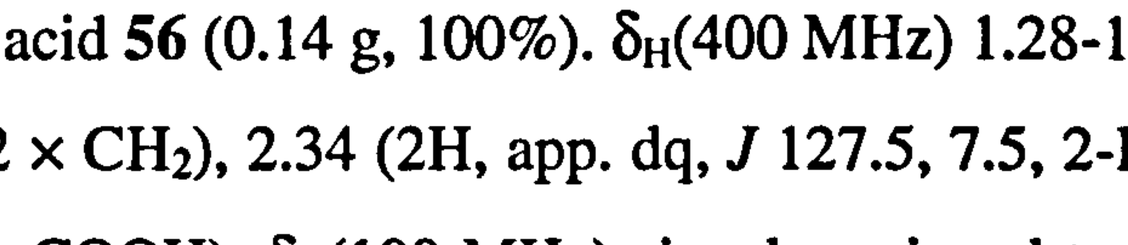
54 41

Ethyl 9-hydroxynonanoate **54** (0.2 g, 0.99 mmol) dissolved in ethanol (1 mL) was added dropwise to sodium hydroxide (2.0 M, 10 mL) and heated to reflux for 24 hours. The mixture was quenched with water (70 mL) and acidified to pH 2 with hydrochloric acid (2.0 M). The solution was then extracted with ethyl acetate (3 × 100 mL), dried over sodium sulfate, filtered and concentrated *in vacuo* to yield acid **41** (0.17 g, 100%) as a white solid. m.p. 42-44 °C (from ethyl acetate/petroleum ether 40-60 °C), lit.[67] 43-44 °C; δ_H(400 MHz) 1.28-1.40 (8H, m, 4 × CH_2), 1.52-1.70 (4H, m, 2 × CH_2), 2.34 (2H, t, *J* 7.5, 2-H_2), 3.65 (2H, t, *J* 6.5, 9-H_2), 6.61 (1H, br s, COOH); δ_C(100 MHz) 24.7 (CH_2), 25.7 (CH_2), 29.0 (CH_2), 29.2 (CH_2), 30.8 (CH_2), 32.5 (CH_2), 34.1 (C-2), 63.0 (C-9), 179.3 (C-1); *m/z* (ESI) 197 (MNa^+, 100%), 185 (20), 123 (11) and 96 (12).

[1,2-$^{13}C_2$]-9-Hydroxynonanoic Acid 56[67]

The above reaction was repeated using [1,2-$^{13}C_2$]-9-hydroxynonanoate **55** (0.16 g, 0.79 mmol) to give acid **56** (0.14 g, 100%). δ_H(400 MHz) 1.28-1.40 (8H, m, 4 × CH_2), 1.50-1.70 (4H, m, 2 × CH_2), 2.34 (2H, app. dq, *J* 127.5, 7.5, 2-H_2), 3.65 (2H, br s, 9-H_2), 7.59 (1H, br s, COOH); δ_C(100 MHz) signals assigned to C-1 (179.3 ppm) and C-2 (34.1 ppm) appear as doublets (*J* 75.0) and show an enhancement of >95% (based on ^{1}H NMR and MS data); *m/z* (CI) 177 (MH^+, 38%), 159 (24), 141 (100), 123 (27), 97 (37), 85 (22), 83 (37) and 69 (19).

S-2-(Acetylamino)ethyl Ethanethioate (*N,S*-Diacetylcysteamine) 240[169]

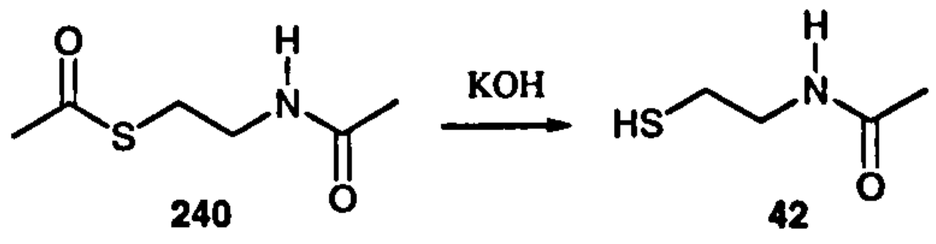


277 240

To a 3-necked flask at 0 °C containing 2-aminoethanethiol hydrochloride **277** (20.00 g, 176.0 mmol) in water (174 mL) was attached two dropping funnels and a pH probe. In one funnel was placed potassium hydroxide (8.0 M, 170 mL) and in the other was placed acetic anhydride (51 mL). The solution was adjusted to pH 8.0 with potassium hydroxide. A slight pink tinge was observed. The acetic anhydride and the potassium

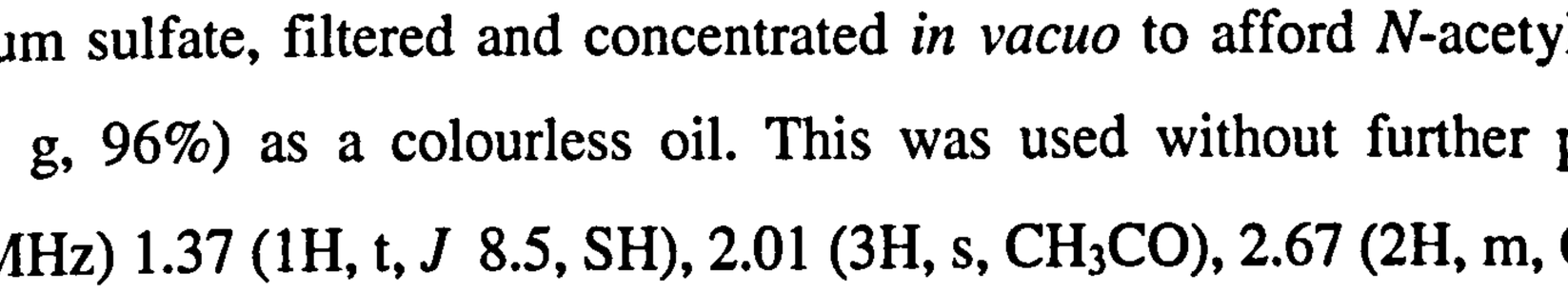
hydroxide were then added dropwise at such a rate that the pH was maintained at approximately 8.0. The solution gradually became colourless. When all the acetic anhydride had been added, the pH of the solution was adjusted to pH 7.0 with hydrochloric acid (2.0 M) and the mixture was stirred at room temperature for 1 hour. The reaction solution was then saturated with sodium chloride and extracted with DCM (4 × 175 mL). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to furnish *N,S*-diacetylcysteamine **240** (26.19 g, 92%) as a colourless oil. This was used without further purification. δ_H(400 MHz) 1.76 (3H, s, CH_3CON), 2.12 (3H, s, CH_3COS), 2.79 (2H, t, *J* 7.0, CH_2S), 3.40 (2H, td, *J* 7.0, 6.0, CH_2N), 7.42 (1H, br s, NH); δ_C(100 MHz) 23.0 (CH_2S), 28.7 (CH_2N), 30.5 (CH_3COS), 39.4 (CH_3CON), 170.3 (CON), 196.1 (COS); *m/z* (EI) 162 (MH^+, 100%), 120 (45), 102 (24), 86 (34), 75 (24) and 59 (26).

S-2-(Acetylamino)ethanethiol (*N*-Acetylcysteamine) 42[169]



N,S-Diacetylcysteamine **240** (1.00 g, 6.2 mmol) in water (15 mL) was cooled to 0 °C. Solid potassium hydroxide (1.05 g, 18.8 mmol) was added and the mixture was stirred under an atmosphere of nitrogen at 0 °C for 50 minutes. The pH was adjusted to 7.0 with hydrochloric acid (2.0 M) and the reaction mixture was saturated with sodium chloride and extracted with DCM (3 × 50 mL). The organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to afford *N*-acetylcysteamine **42** (0.71 g, 96%) as a colourless oil. This was used without further purification. δ_H(400 MHz) 1.37 (1H, t, *J* 8.5, SH), 2.01 (3H, s, CH_3CO), 2.67 (2H, m, CH_2S), 3.44 (2H, td, *J* 8.5, 6.5, CH_2N), 6.01 (1H, br s, NH); δ_C(100 MHz) 23.3 (CH_2N), 24.7 (CH_2S), 42.5 (CH_3CON), 170.2 (CON); *m/z* (EI) 119 (M^+, 48%), 88 (11), 86 (64), 84 (71), 75 (7), 71 (29) and 59 (100).

9-Hydroxynonanoic Acid *N*-Acetylcysteamine Thiol Ester 43[23,67]

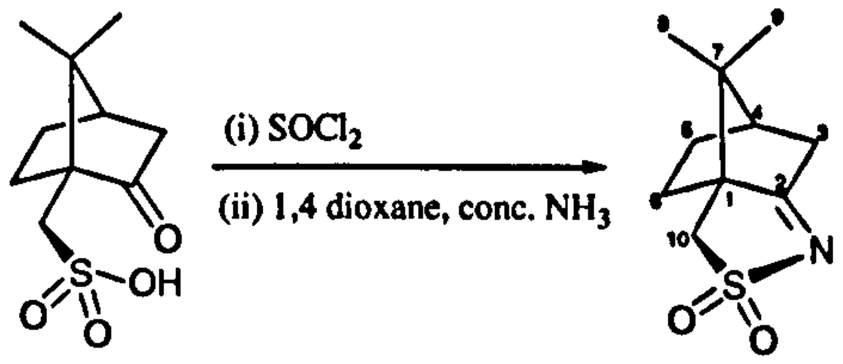


Freshly prepared *N*-acetylcysteamine **42** (0.43 g, 3.60 mmol) in DCM (50 mL) was stirred at 0 °C under an atmosphere of nitrogen. DCC (0.58 g, 2.80 mmol) in DCM (1 mL) was added followed by DMAP (0.03 g, 0.20 mmol) in DCM (1 mL). This was stirred for 10 minutes before addition of 9-hydroxynonanoic acid **41** (0.43 g, 2.30 mmol). The solution was left at 0 °C for 2 hours and was allowed to warm to room temperature overnight. The reaction was subsequently quenched with saturated ammonium chloride (100 mL) and was extracted with DCM (3 × 100 mL). The combined organic extracts were dried over magnesium sulfate, and copper sulfate impregnated silica (prepared by stirring flash silica in a saturated solution of copper sulfate for 10 minutes and then drying *in vacuo* to a free flowing powder)[170] was added to remove any unreacted *N*-acetylcysteamine **42**. The solution was then filtered and concentrated *in vacuo* to yield a white solid. Ethyl acetate was added (3 mL) to dissolve the product and the insoluble urea by-product was then filtered off. Evaporating off the filtrate *in vacuo* furnished the thiol ester **43** (0.40 g, 59%) as a white powdery solid. m.p. 63-64 °C (from ethyl acetate/petroleum ether 40-60 °C), (previously characterised as an oil)[67]; δ_H(400 MHz) 1.23-1.40 (8H, m, 4 × CH_2), 1.50-1.76 (4H, m, 2 × CH_2), 1.97 (3H, s, CH_3), 2.57 (2H, t, *J* 7.5, 2-H_2), 3.03 (2H, t, *J* 6.5, CH_2S), 3.44 (2H, app. q, *J* 6.5, C*H*$_2$NH), 3.64 (2H, t, *J* 6.5, 9-H_2), 5.84 (1H, br s, NH); δ_C(100 MHz) 23.3 (CH_3), 25.6 (CH_2), 28.5 (CH_2), 28.8 (CH_2), 29.1 (2 coincident signals, 2 × CH_2), 32.7 (CH_2), 34.0 (CH_2), 39.8 (CH_2N), 44.1 (C-2), 63.0 (C-9), 170.3 (CON), 200.3 (C-1); *m/z* (ESI) 298 (MNa^+, 100%) and 215 (30).

[1,2-$^{13}C_2$]-9-Hydroxynonanoic Acid *N*-Acetylcysteamine Thiol Ester 1

The above reaction was repeated using acid **56** (0.17 g, 0.95 mmol) to give *thiol ester* **1** (0.08 g, 30%). δ_H(400 MHz) 1.24-1.38 (8H, m, 4 × CH_2), 1.52-1.72 (4H, m, 2 × CH_2), 1.97 (3H, s, CH_3), 2.57 (2H, ddt, *J* 129.0, 7.5, 6.5, 2-H_2), 3.02 (2H, dt, *J* 6.5, 5.0, CH_2S), 3.43 (2H, app. q, *J* 6.5, C*H*$_2$NH), 3.63 (2H, t, *J* 6.5, 9-H_2), 6.09 (1H, br s, NH); δ_C(100 MHz) signals assigned to C-1 (200.3 ppm) and C-2 (44.1 ppm) appear as doublets (*J* 75.0) and show an enhancement of >95% (based on ^{1}H NMR and MS data); *m/z* (ESI) 300 (MNa^+, 12%), 278 (100), 225 (34), 145 (6) and 101 (12).

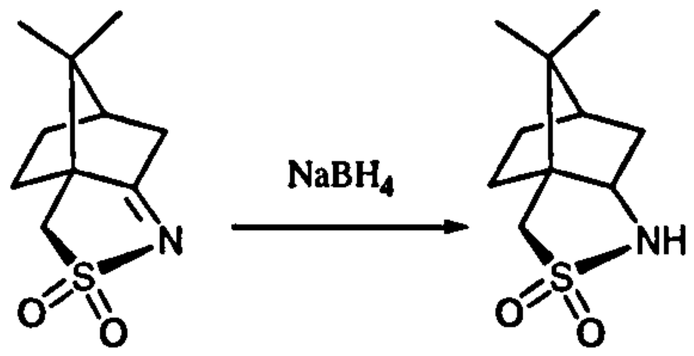
(1*S*)-1,10-(Camphorsulfonyl)imine 279[171]



278 279

Thionyl chloride (38.0 mL, 516.4 mmol) was added dropwise to (1*S*)-10-camphorsulfonic acid **278** (20.00 g, 86.00 mmol) and the mixture was heated to reflux for 4 hours. Once cooled, toluene (100 mL) was added and the remaining thionyl chloride was evaporated off *in vacuo* azeotroping with the toluene. The resulting orange oil was dissolved in 1,4-dioxane (50 mL) and added dropwise to concentrated ammonia solution (300 mL) at 0 °C. The solution was then stirred at room temperature for 14 hours. The resulting precipitate was filtered off and washed with water (2 × 20 mL) to yield (1*S*)-1,10-(camphorsulfonyl)imine **279** as an off-white solid (17.78 g, 97%). This was used without further purification. $[\alpha]_D^{25}$ -32.0 (*c* 1.00, $CHCl_3$), lit.[171] $[\alpha]_D$ -32.7 (*c* 1.89, $CHCl_3$); m.p. 235-237 °C, lit.[171] 225-228 °C; δ_H(400 MHz) 0.87 and 1.09 (each 3H, each s, 8-H_3 and 9-H_3), 1.48 and 1.78 (each 1H, each t, *J* 9.0, 5-*H*H and 6-*H*H), 2.02-2.12 (2H, m, 5-H*H* and 6-H*H*), 2.26 (1H, t, *J* 4.5, 4-H), 2.39 (1H, d, *J* 19.5, 3-*H*H), 2.78 (1H, ddd, *J* 19.5, 4.5, 2.0, 3-H*H*), 2.98 (1H, d, *J* 13.0, 10-H*H*), 3.19 (1H, d, *J* 13.0, 10-*H*H); δ_C(100 MHz) 19.0 and 19.4 (C-8 and C-9), 26.6 and 28.4 (C-5 and C-6), 35.9 (C-3), 44.6 (C-4), 48.0 (C-7), 49.4 (C-10), 64.5 (C-1), 195.4 (C-2); *m/z* (CI) 214 (MH^+, 100%), 150 (14), 134 (8), 109 (25), 108 (23) and 93 (6).

(1*S*,2*R*)-Bornane-10,2-camphorsultam 76[171]

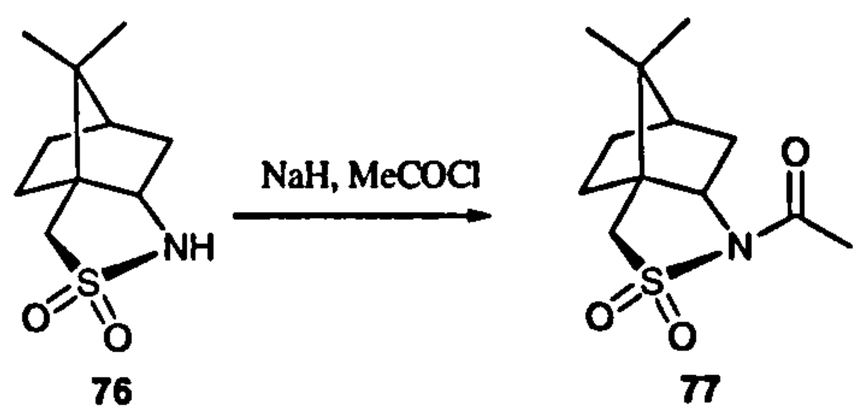


279 76

(1*S*)-1,10-(Camphorsulfonyl)imine **279** (7.98 g, 37.1 mmol) was suspended in methanol (85 mL) and water (15 mL) and cooled to 0 °C. Sodium borohydride (3.46 g, 91.4 mmol) was added portionwise and the reaction was stirred at room temperature for 4 hours. The methanol was evaporated *in vacuo* and the resulting residue was suspended in DCM (70 mL) and acidified with sulfuric acid (1.0 M, 60

mL). The organic layer was separated and the aqueous layer was extracted with DCM (3 × 50 mL). The organic layers were combined, washed with brine (50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The resulting solid was recrystallised from ethanol to furnish camphorsultam **76** (7.11 g, 88%) as an off white solid. $[\alpha]_D^{25}$ -32.0 (*c* 1.00, $CHCl_3$), lit.[171] $[\alpha]_D$ -30.5 (*c* 4.00, $CHCl_3$); m.p. 182-184 °C, lit.[171] 183-184 °C; δ_H(400 MHz) 0.94 and 1.13 (each 3H, each s, 8-H_3 and 9-H_3), 1.23-1.51 (2H, m, 5-*H*H and 6-*H*H), 1.83-2.02 (5H, m, 3-H_2, 4-H, 5-H*H* and 6-H*H*), 3.10 (1H, d, *J* 13.5, 10-*H*H), 3.14 (1H, d, *J* 13.5, 10-H*H*), 3.43 (1H, m, 2-H), 4.16 (1H, br s, NH); δ_C(100 MHz) 20.5 and 20.5 (C-8 and C-9), 26.8 and 31.9, (C-5 and C-6), 36.1 (C-3), 44.7 (C-4), 47.5 (C-7), 50.4 (C-10), 55.1 (C-1), 62.9 (C-2); *m/z* (CI) 216 (MH^+, 100%), 199 (13), 151 (47), 135 (82), 119 (19), 109 (34), 93 (20) and 84 (28).

(1*S*,2*R*)-*N*-Acetyl-bornane-10,2-sultam 77[172]

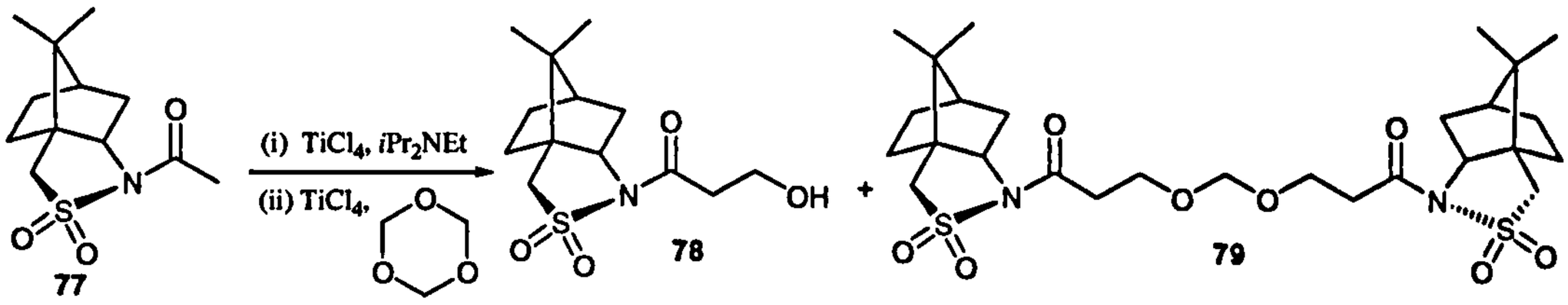


76 **77**

(1*S*)-(-)-Camphorsultam **76** (1.50 g, 7.00 mmol) in dry toluene (5 mL) was added dropwise to a stirred suspension of sodium hydride (60% dispersion in oil prewashed with petrol, 0.95 g, 24.0 mmol) in dry toluene (40 mL) at room temperature for 1 hour. Acetyl chloride (0.50 mL, 7.00 mmol) was added dropwise and the mixture was stirred for 2 hours at room temperature. The reaction was quenched with ammonium chloride (10 mL) and left to stir for a further 10 minutes. The organic layer was then separated and the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield sultam **77** (1.73 g, 97%) as a white solid. m.p. 135-136 °C, lit.[172] 130-132 °C; $[\alpha]_D^{22}$ -117.0 (*c* 3.50, CH_2Cl_2), $[\alpha]_D$ lit.[172] -116.1 (*c* 3.20, CH_2Cl_2); δ_H(400 MHz) 0.97 and 1.16 (each 3H, each s, 8-H_3 and 9-H_3), 1.30-1.45 (2H, m, 5-*H*H and 6-*H*H), 1.83-1.97 (3H, m, 4-H, 5-H*H* and 6-H*H*), 2.08 (1H, dd, *J* 14.0, 8.0, 3-*H*H), 2.12-2.20 (1H, m, 3-H*H*), 2.41 (3H, s, 2′-H_3), 3.44 (1H, d, *J* 14.0, 10-*H*H), 3.51 (1H, d, *J* 14.0, 10-H*H*), 3.86 (1H, dd, *J* 8.0, 5.0, 2-H); δ_C(100 MHz) 19.8 and 20.8 (C-

8 and C-9), 23.2 (C-2′), 26.5 and 32.9 (C-5 and C-6), 38.5 (C-3), 44.8 (C-4), 47.8 and 48.4 (C-7 and C-1), 52.9 (C-10), 65.3 (C-2), 168.6 (C-1′).

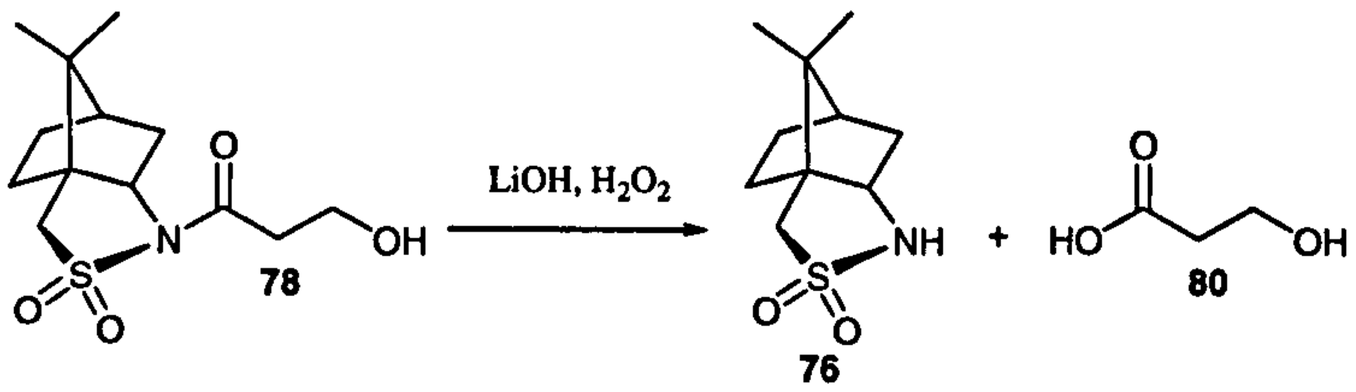
(1*S*,2*R*)-*N*-(3′-Hydroxypropionyl)-bornane-10,2-sultam 78



A solution of titanium (IV) chloride (1.0 M in DCM, 3.8 mL, 3.8 mmol) was added to a stirred solution of acetylsultam **77** (0.50 g, 1.94 mmol) in dry DCM (11 mL) at -78 °C under nitrogen. After 10 minutes ethyldiisopropylamine (0.66 mL, 3.8 mmol) was added, followed 1 hour later by trioxane (0.18 g, 1.98 mmol) and titanium (IV) chloride (1.0 M in DCM, 3.8 mL, 3.8 mmol). The reaction was maintained at -78 °C for 6 hours and was then allowed to warm to room temperature overnight. The solution was extracted with DCM (3 × 50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield a yellow oil. Purification by flash column chromatography (SiO_2, 20% ethyl acetate/petroleum ether 40-60 °C) afforded the desired *alcohol* **78** (0.16 g, 29% (isolated yield)) as well as a 1:3 mixture of **78** and a *dimer* side-product **79** (0.13 g). Despite several attempts, it was never possible to separate more than an analytical quantity of the latter compound. *Alcohol* **78**: Found: C, 54.52; H, 7.37; N, 4.96; S, 10.83; $C_{13}H_{21}NO_4S$ requires C, 54.33; H, 7.37; N, 4.87; S, 11.16; m.p. 140 °C (from chloroform); $[\alpha]_D^{23}$ -110.13 (*c* 1.05, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3549 (OH, H-bonded), 2952, 2922, 2882, 1683 (C=O), 1318, 1065, 770; δ_H(400 MHz) 0.98 and 1.16 (each 3H, each s, 8-H_3 and 9-H_3), 1.20-1.48 (2H, m, 5-*H*H and 6-*H*H), 1.84-1.98 (3H, m, 4-H, 5-H*H* and 6-H*H*), 2.08 (1H, dd, *J* 14.0, 8.0, 3-H*H*), 2.18 (1H, m, 3-*H*H), 2.51 (1H, t, *J* 6.5, OH), 2.99 (2H, t, *J* 5.5, 2′-H_2), 3.45 (1H, d, *J* 14.0, 10-*H*H), 3.52 (1H, d, *J* 14.0, 10-H*H*), 3.85-3.97 (3H, m, 2-H and 3′-H_2); δ_C(100 MHz) 19.9 and 20.8 (C-8 and C-9), 26.5 and 32.9 (C-5 and C-6), 37.9 (C-2′), 38.4 (C-3), 44.7 (C-4), 47.8 and 48.6 (C-1 and C-7), 52.9 (C-10), 58.1 (C-3′), 65.2 (C-2), 171.4 (C-1′); *m/z* (CI) 288 (MH^+, 21%), 270 (12), 216 (100), 151 (8), 135 (37) and 84 (9); Found (CI): 288.1261 (MH^+), ($C_{13}H_{22}NO_4S$ requires 288.1270). *Dimer* **79**: Found: C, 55.39; H, 7.37; N, 4.86; S, 10.72; $C_{27}H_{42}N_2O_8S_2$ requires C, 55.27; H, 7.21; N, 4.77; S, 10.93; m.p. 159-160 °C (from chloroform); $[\alpha]_D^{22}$ -101.46 (*c* 3.43, $CHCl_3$);

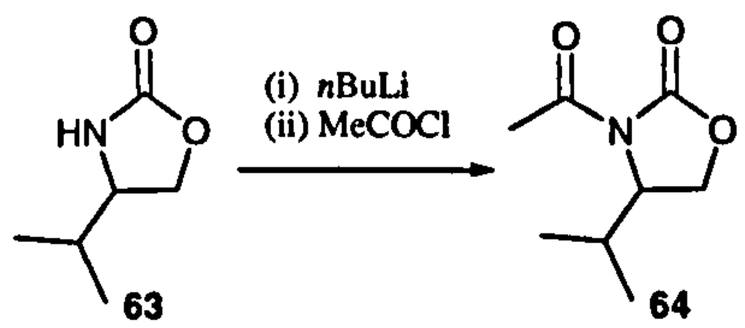
ν_{max}(neat)/cm^{-1} 2954, 2893, 1687 (C=O), 1325, 1278, 1167, 1132, 1119, 1021, 777; δ_H(400 MHz) 0.97 and 1.16 (each 6H, each s, 2 × 8-H_3 and 9-H_3), 1.30-1.45 (4H, m, 2 × 5-*H*H and 2 × 6-*H*H), 1.82-1.97 (6H, m, 2 × 4-H, 2 × 5-H*H* and 2 × 6-H*H*), 2.07 (2H, dd, *J* 13.5, 7.5, 2 × 3-*H*H), 2.11-2.20 (2H, m, 2 × 3-H*H*), 2.89-3.11 (4H, m, 2 × 2′-H_2), 3.43 (2H, d, *J* 14.0, 2 × 10-*H*H), 3.50 (2H, d, *J* 14.0, 2 × 10-H*H*), 3.79-3.93 (6H, m, 2 × 2-H and 2 × 3′-H_2), 4.67 (2H, s, OCH_2O); δ_C(100 MHz) 19.9 and 20.8 (2 × C-8 and 2 × C-9), 26.5 and 32.8 (2 × C-5 and 2 × C-6), 35.9 (2 × C-2′), 38.4 (2 × C-3), 44.7 (2 × C-4), 47.8 and 48.5 (2 × C-1 and 2 × C-7), 52.9 (2 × C-10), 62.5 (2 × C-3′), 65.2 (2 × C-2), 95.1 (OCH_2O) and 169.6 (2 × C-1′); *m/z* (ESI) 609 (MNa^+, 100%), 387 (6), 380 (7), 300 (37) and 203 (11); Found (ESI): 609.2275 (MNa^+), ($C_{27}H_{42}N_2O_8S_2Na$ requires 609.2275).

3-Hydroxypropanoic Acid 80[67]



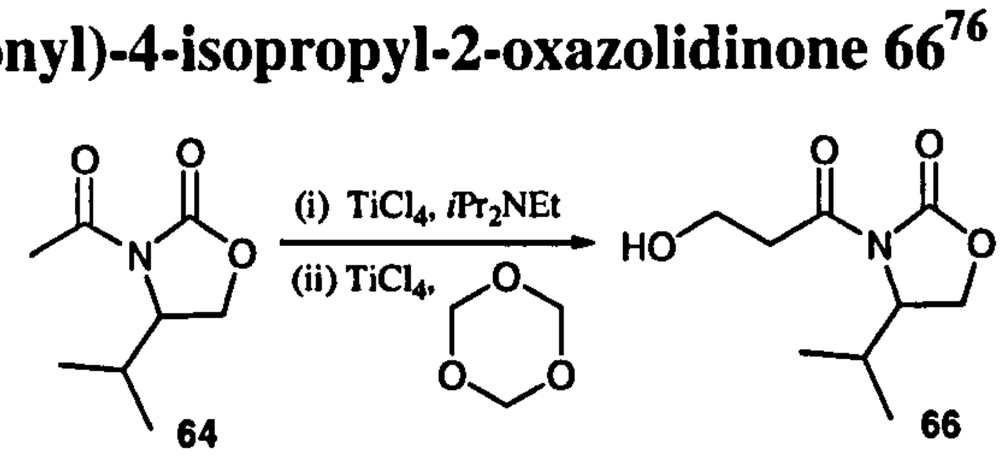
Lithium hydroxide monohydrate (0.024 g, 0.58 mmol) and hydrogen peroxide solution (30% solution in water, 0.10 mL, 0.87 mmol) were added successively to a solution of sultam **78** (0.085 g, 0.29 mmol) in THF (3 mL) and water (1 mL) at 0 °C. The mixture was stirred at room temperature overnight. A white precipitate formed. The reaction was again cooled to 0 °C and quenched with sodium sulfite solution (1.45 mL, 0.87 mmol). After 10 minutes the pH was adjusted to 10 with saturated aqueous sodium hydrogen carbonate. The organic solvent was removed *in vacuo* and then water (5 mL) was added and the solution was extracted with DCM (3 × 30 mL) to retrieve auxiliary **76**. The aqueous layer was acidified to pH 2 with hydrochloric acid (6.0 M) and extracted with diethyl ether (4 × 50 mL). The combined layers were dried over sodium sulfate, filtered and concentrated *in vacuo* to yield the acid **80** (0.027 g, 100%) as a colourless oil. δ_H(400 MHz, D_2O) 2.61 (2H, t, *J* 6.0, 2-H_2), 3.85 (2H, t, *J* 6.0, 3-H_2); δ_C(100 MHz, D_2O) 39.4 (C-2), 60.0 (C-3), 179.2 (C-1).

N-Acetyl-4-isopropyl-2-oxazolidinone 64[89]



A solution of oxazolidinone **63** (5.00 g, 38.71 mmol) in dry THF (80 mL) was cooled to -10 °C under an atmosphere of nitrogen and treated with *n*-butyllithium (2.5 M in hexanes, 24.78 mL, 61.94 mmol). After 30 minutes, acetyl chloride (9.6 mL, 135.49 mmol) was added dropwise. The mixture was allowed to warm to room temperature overnight and was then quenched with water (50 mL). The mixture was extracted with ethyl acetate (3 × 75 mL) and the combined organic layers were then washed with saturated sodium hydrogen carbonate solution (20 mL) and water (20 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to afford a yellow/orange oil. Purification by flash column chromatography (SiO_2, 30% ethyl acetate/petroleum ether 40-60 °C) furnished the acylated oxazolidinone **64** (6.05 g, 91%) as a yellow oil. δ_H(400 MHz) 0.88 and 0.92 (each 3H, each d, *J* 7.0, $CH(CH_3)_2$), 2.40 (1H, app. quint.d, *J* 7.0, 4.0, $CH(CH_3)_2$), 2.54 (3H, s, CH_3CO), 4.22 (1H, dd, *J* 9.5, 3.0, 5-*H*H), 4.27 (1H, dd, *J* 9.5, 8.5, 5-H*H*), 4.43 (1H, br dt, *J* 8.5, 3.5, 4-H); δ_C(100 MHz) 14.6 ($CHCH_3$), 18.0 ($CHCH_3$), 23.8 (CH_3CO), 28.3 ($CH(CH_3)_2$), 58.4 (C-4), 63.3 (C-5), 154.3 (C-2), 170.3 (CH_3CO).

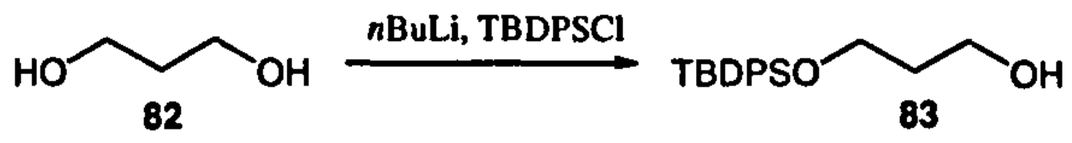
N-(3′-Hydroxypropionyl)-4-isopropyl-2-oxazolidinone 66[76]



A solution of titanium (IV) chloride (1.0 M in DCM, 69.4 mL, 69.4 mmol) was added to a stirred solution of oxazolidinone **64** (6.05 g, 35.34 mmol) in dry DCM (150 mL) at -78 °C under an atmosphere of nitrogen. After 10 minutes ethyldiisopropylamine (12.5 mL, 71.50 mmol) was added, followed 1 hour later by trioxane (3.26 g, 36.10 mmol) and titanium (IV) chloride (1.0 M in DCM, 69.4 mL, 69.4 mmol). The reaction was maintained at -78 °C for 6 hours and was then allowed to warm to room temperature overnight. Saturated aqueous ammonium chloride solution (150 mL) was added and the solution was extracted with DCM (3 × 150 mL), dried over magnesium

sulfate, filtered and concentrated *in vacuo* to yield the crude product (9.55 g) as a yellow oil. After several attempts, purification by flash column chromatography (SiO_2, 20-30% ethyl acetate/petroleum ether 40-60 °C) failed to separate the mixture of products but did provide a small sample of the desired alcohol **66** as a colourless oil. δ_H(400 MHz) 0.89 and 0.94 (each 3H, each d, *J* 7.0, CH(C*H*$_3$)$_2$), 1.55 (1H, br s, OH), 2.39 (1H, m, C*H*(CH$_3$)$_2$), 3.08 and 3.16 (each 1H, each ddd, *J* 18.0, 6.0, 4.5, 2′-H$_2$), 3.94 (1H, m, 3′-H$_2$), 4.24 (1H, dd, *J* 9.0, 3.0, 5-*H*H), 4.30 (1H, dd, *J* 9.0, 8.0, 5-H*H*), 4.45 (1H, ddd, *J* 8.0, 4.0, 3.0, 4-H); δ_C(100 MHz) 14.7 (CH(*C*H$_3$)$_2$), 18.0 (CH(*C*H$_3$)$_2$), 28.4 (*C*H(CH$_3$)$_2$), 38.3 (C-2′), 58.1 and 58.4 (C-3′ and C-4), 63.6 (C-5), 154.1 (C-2), 172.8 (C-1′).

3-(*tert*-Butyldiphenylsilyloxy)propanol 83[103,173]



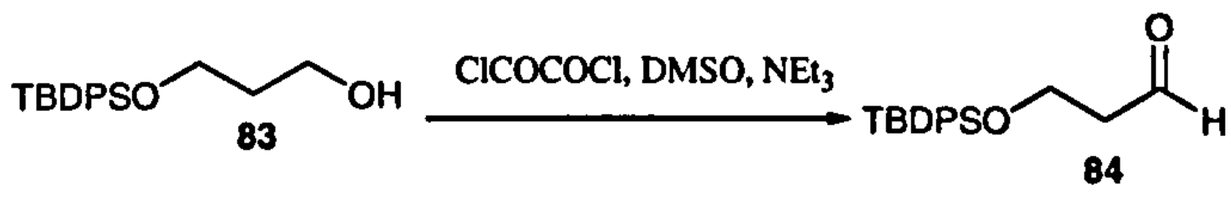
n-Butyllithium (2.5 M in hexanes, 18.5 mL, 46.25 mmol) was added to a solution of propane-1,3-diol **82** (3.32 mL, 46.00 mmol) in dry THF (75 mL) at -78 °C under an atmosphere of nitrogen. *tert*-Butylchlorodiphenylsilane (11.96 mL, 46.00 mmol) was then added and the mixture was stirred at -78 °C for 15 minutes, followed by 15 minutes at room temperature and then refluxed for 21 hours. The solvent was removed *in vacuo* to give a white oily suspension. Purification by flash column chromatography (SiO_2, 5-10% ethyl acetate/petroleum ether 40-60 °C) yielded silyl ether **83** (12.31 g, 85%) as a colourless oil which crystallised upon trituration. m.p. 38-40 °C, lit.[103] 36-39 °C; δ_H(400 MHz) 1.06 (9H, s, SiC(C*H*$_3$)$_3$), 1.81 (2H, app. quint., *J* 5.5, 2-H$_2$), 2.45 (1H, t, *J* 5.5, OH), 3.81-3.87 (4H, m, 1-H$_2$ and 3-H$_2$), 7.36-7.46 (6H, m, 6 × Ar-H), 7.66-7.71 (4H, m, 4 × Ar-H); δ_C(100 MHz) 19.1 (Si*C*(CH$_3$)$_3$), 26.8 (SiC(*C*H$_3$)$_3$), 34.3 (C-2), 61.9 and 63.3 (C-1 and C-3), 127.8 (4 × Ar-C), 129.8 (2 × Ar-C), 133.3 (2 × Ar-C$_{ipso}$), 135.6 (4 × Ar-C).

[1,1,3,3-^{2}H$_4$]-3-(*tert*-Butyldiphenylsilyloxy)propanol 88

The above reaction was repeated using deuterated diol **87** (0.63 g, 7.86 mmol) to furnish *silyl ether* **88** (2.30 g, 92%) as a colourless oil which crystallised upon cooling. δ_H(400 MHz) 1.05 (9H, s, SiC(C*H*$_3$)$_3$), 1.78 (2H, s, 2-H$_2$), 2.36 (1H, s, OH), 7.37-7.46 (6H, m, 6 × Ar-H), 7.66-7.70 (4H, m, 4 × Ar-H); δ_C(100 MHz) 19.1

($SiC(CH_3)_3$), 26.8 ($SiC(CH_3)_3$), 33.8 (C-2), 61.2 and 62.5 (each quint., *J* 21.5, C-1 and C-3), 127.7 (4 × Ar-C), 129.8 (2 × Ar-C), 133.3 (2 × Ar-C_{ipso}), 135.5 (4 × Ar-C); *m/z* (CI) 319 (MH^+, 6%), 261 (25), 241 (21), 199 (39), 183 (62), 163 (30), 121 (100), 93 (21) and 75 (13).

3-(*tert*-Butyldiphenylsilyloxy)propanal 84[173]

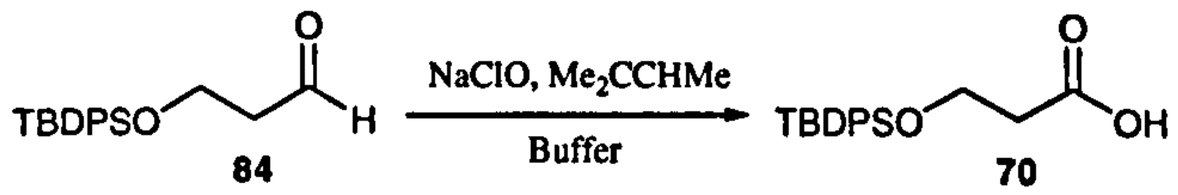


Dimethylsulfoxide (1.8 mL, 25.31 mmol) was added dropwise to oxalyl chloride (1.1 mL, 12.66 mmol) in dry DCM (60 mL) at -78 °C under nitrogen. After 1 hour alcohol **83** (2.00 g, 6.36 mmol) in dry DCM (10 mL) was added dropwise. An hour later triethylamine (7.1 mL, 50.63 mmol) was added and after 20 minutes the mixture was allowed to warm to room temperature. Water (75 mL) was then added to quench the reaction. The layers were separated and the aqueous layer was extracted with DCM (3 × 100 mL) The combined organic extracts were washed with hydrochloric acid (2.0 M, 50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to afford a yellow oil. Purification by flash column chromatography (SiO_2, 10% ethyl acetate/petroleum ether 40-60 °C) furnished aldehyde **84** (1.90 g, 95%) as a very pale yellow oil. δ_H(400 MHz) 1.04 (9H, s, $SiC(CH_3)_3$), 2.59 (2H, dt, *J* 6.0, 2.0, 2-H_2), 4.01 (2H, t, *J* 6.0, 3-H_2), 7.34-7.45 (6H, m, 6 × Ar-H), 7.64-7.73 (4H, m, 4 × Ar-H), 9.80 (1H, t, *J* 2.0, 1-H); δ_C(100 MHz) 19.1 ($SiC(CH_3)_3$), 26.7 ($SiC(CH_3)_3$), 46.4 (C-2), 58.3 (C-3), 127.8 (4 × Ar-C), 129.8 (2 × Ar-C), 134.8 (2 × Ar-C_{ipso}), 135.5 (4 × Ar-C), 201.9 (C-1).

[1,3,3-$^2H_3$]-3-(*tert*-Butyldiphenylsilyloxy)propanal 89

The above reaction was repeated using deuterated alcohol **88** (2.30 g, 7.22 mmol) to afford *aldehyde* **89** (1.84 g, 81%) as a pale yellow oil. δ_H(400 MHz) 1.05 (9H, s, $SiC(CH_3)_3$), 2.59 (2H, s, 2-H_2), 7.36-7.47 (6H, m, 6 × Ar-H), 7.63-7.69 (4H, m, 4 × Ar-H); δ_C(100 MHz) 19.1 ($SiC(CH_3)_3$), 26.7 ($SiC(CH_3)_3$), 46.0 (C-2), 57.6 (quint., *J* 21.5, C-3), 127.7 (4 × Ar-C), 129.8 (2 × Ar-C), 133.2 (2 × Ar-C_{ipso}), 135.5 (4 × Ar-C), 201.6 (t, *J* 26.0, C-1); *m/z* (ESI) 338 (MNa^+, 100%) and 123 (70).

3-(*tert*-Butyldiphenylsilyloxy)propanoic acid 70[76]

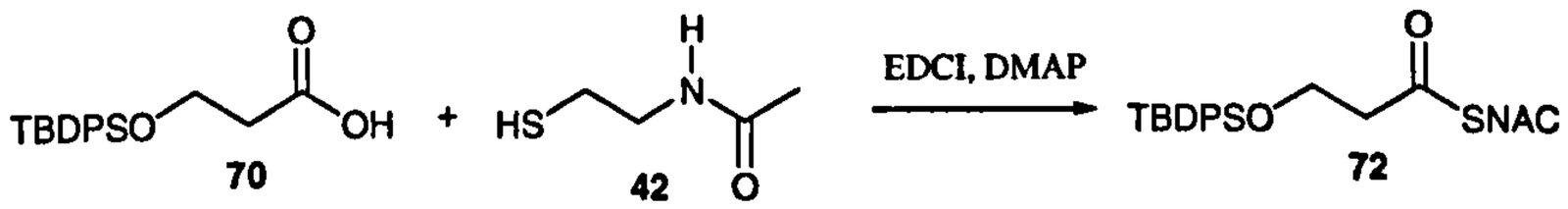


Aldehyde **84** (0.50 g, 1.60 mmol) was dissolved in *tert*-butanol (20 mL) and 2-methylbut-2-ene (9 mL). A solution of sodium chlorite (1.72 g, 19.00 mmol) and sodium dihydrogen phosphate (1.44 g, 1.20 mmol) in water (10 mL) was then added. The mixture was stirred vigorously for 1 hour and was then extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were dried over magnesium sulfate and concentrated *in vacuo*. Chloroform (2 × 40 mL) was added to azeotrope with the remaining *tert*-butanol furnishing the acid **70** (0.53 g, 100%) as a white solid. No further purification was required, however a small sample was recrystallised from ethanol for characterisation. m.p. 104-106 °C (from ethanol), (previously characterised as an oil)[76]; δ_H(400 MHz) 1.04 (9H, s, $SiC(CH_3)_3$), 2.60 (2H, t, *J* 6.0, 2-H_2), 3.94 (2H, t, *J* 6.0, 3-H_2), 7.35-7.45 (6H, m, 6 × Ar-H), 7.65-7.73 (4H, m, 4 × Ar-H); δ_C(100 MHz) 19.2 ($SiC(CH_3)_3$), 26.7 ($SiC(CH_3)_3$), 37.5 (C-2), 59.6 (C-3), 127.8 (4 × Ar-C), 129.8 (2 × Ar-C), 134.8 (2 × Ar-C_{ipso}), 135.6 (4 × Ar-C), 177.3 (C-1).

[3,3-$^2H_2$]-3-(*tert*-Butyldiphenylsilyloxy)propanoic acid 90

The above reaction was repeated using deuterated aldehyde **89** (1.84 g, 5.85 mmol) to furnish *acid* **90** (1.93 g, 100%) as a white solid. δ_H(400 MHz) 1.04 (9H, s, $SiC(CH_3)_3$), 2.59 (2H, s, 2-H_2), 7.34-7.46 (6H, m, 6 × Ar-H), 7.64-7.69 (4H, m, 4 × Ar-H); δ_D(400 MHz, $CHCl_3$) 3.96 (2D, s, 3-D_2); δ_C(100 MHz) 19.4 ($SiC(CH_3)_3$), 27.0 ($SiC(CH_3)_3$), 37.6 (C-2), 59.1 (quint., *J* 21.5, C-3), 127.9 (4 × Ar-C), 129.9 (2 × Ar-C), 133.4 (2 × Ar-C_{ipso}), 135.7 (4 × Ar-C), 177.6 (C-1); *m/z* (CI) 331 (MH^+, 13%), 313 (12), 273 (45), 253 (100), 199 (16), 85 (17) and 83 (25).

3-(*tert*-Butyldiphenylsilyloxy)propanoic Acid *N*-Acetylcysteamine Thiol Ester 72[76]



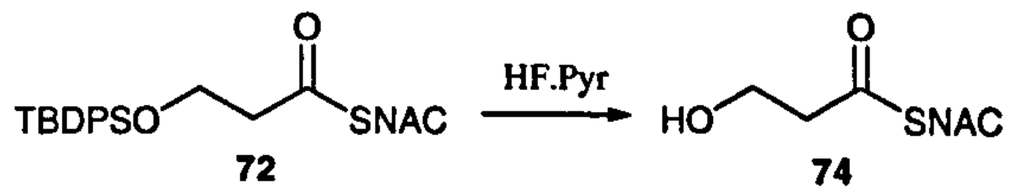
Acid **70** (0.52 g, 1.59 mmol) was dissolved in DCM (25 mL) at 0 °C. EDCI (0.34 g, 1.84 mmol) and DMAP (0.024 g, 0.12 mmol) in DCM (1 mL) were added, followed by *N*-acetylcysteamine **42** (freshly prepared, 0.27 g, 2.28 mmol) in DCM (1 mL) 10

minutes later. The solution was allowed to warm to room temperature overnight and was then quenched with saturated ammonium chloride solution (50 mL). The layers were separated and the aqueous layer was extracted with DCM (3 × 50 mL). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to give a pale yellow oil. Purification by flash column chromatography (SiO_2 topped with a layer of copper sulfate impregnated silica prepared by stirring flash silica in a saturated solution of copper sulfate for 10 minutes and then drying *in vacuo* to a free flowing powder),[170] 50-60% ethyl acetate/petroleum ether 40-60 °C) furnished thiol ester **72** (0.45 g, 66%) as a white solid. m.p. 84-86 °C (from chloroform), (previously characterised as an oil)[76]; δ_H(400 MHz) 1.04 (9H, s, $SiC(CH_3)_3$), 1.92 (3H, s, CH_3CON), 2.79 (2H, t, *J* 6.0, 2-H_2), 3.04 (2H, t, *J* 6.5, CH_2S), 3.43 (2H, td, *J* 6.5, 6.0, CH_2N), 3.97 (2H, t, *J* 6.0, 3-H_2), 5.99 (1H, br s, NH), 7.36-7.46 (6H, m, 6 × Ar-H), 7.64-7.67 (4H, m, 4 × Ar-H); δ_C(100 MHz) 19.2 ($SiC(CH_3)_3$), 23.1 (CH_3CON), 26.7 ($SiC(CH_3)_3$), 28.6 (CH_2S), 39.6 (CH_2N), 46.9 (C-2), 60.0 (C-3), 127.7 (4 × Ar-C), 129.8 (2 × Ar-C), 133.2 (2 × Ar-C_{ipso}), 135.5 (4 × Ar-C), 170.3 (CON), 198.2 (C-1).

[3,3-$^2H_2$]-3-(*tert*-Butyldiphenylsilyloxy)propanoic Acid *N*-Acetylcysteamine Thiol Ester 91

The above reaction was repeated using deuterated acid **90** (1.84 g, 5.57 mmol) to afford *thiol ester* **91** (1.89 g, 79%) as a white solid. δ_H(400 MHz) 1.04 (9H, s, $SiC(CH_3)_3$), 1.93 (3H, s, CH_3CON), 2.77 (2H, s, 2-H_2), 3.04 (2H, t, *J* 6.5, CH_2S), 3.40 (2H, td, *J* 6.5, 6.0, CH_2N), 6.76 (1H, br t, *J* 6.0, NH), 7.33-7.44 (6H, m, 6 × Ar-H), 7.63-7.70 (4H, m, 4 × Ar-H); δ_D(400 MHz, $CHCl_3$) 3.93 (2D, s, 3-D_2); δ_C(100 MHz) 18.8 ($SiC(CH_3)_3$), 22.7 (CH_3CON), 26.4 ($SiC(CH_3)_3$), 28.2 (CH_2S), 39.1 (CH_2N), 46.4 (C-2), 59.1 (quint., *J* 21.5, C-3), 127.4 (4 × Ar-C), 129.5 (2 × Ar-C), 132.9 (2 × Ar-C_{ipso}), 135.1 (4 × Ar-C), 170.3 (CON), 197.5 (C-1); *m/z* (ESI) 454 (MNa^+, 100%), 354 (7) and 151 (10); Found (ESI): 454.1821 (MH^+), ($C_{23}H_{29}D_2NO_3SSiNa$ requires 454.1812).

3-Hydroxypropanoic Acid *N*-Acetylcysteamine Thiol Ester 74[76]



Hydrogen fluoride pyridine complex (65-70% hydrogen fluoride, 0.2 mL, ≈1.16 mmol) was added to a solution of thiol ester **72** (0.50 g, 1.16 mmol) in dry THF (25 mL) at 0 °C under nitrogen. The reaction was stirred for 4 hours and then quenched with water (20 mL). The organic layer was removed and the aqueous layer was extracted with DCM (3 × 50 mL). The combined organic layers were dried, filtered and concentrated *in vacuo*. The crude product was then purified by flash column chromatography (SiO_2, 100% ethyl acetate) to give thiol ester **74** (0.08 g, 38%) as a white solid. m.p. 76-78 °C (from chloroform), (previously characterised as an oil)[76]; δ_H(400 MHz) 1.98 (3H, s, CH_3), 2.84 (2H, t, *J* 5.5, 2-H_2), 3.06 (2H, t, *J* 6.0, CH_2S), 3.47 (2H, app. q, *J* 6.0, CH_2N), 3.93 (2H, t, *J* 5.5, 3-H_2), 6.00 (1H, br s, NH); δ_C(100 MHz) 23.2 (CH_3), 28.8 (CH_2S), 39.2 (CH_2N), 46.6 (C-2), 58.8 (C-3), 170.7 (CON), 199.3 (C-1).

[3,3-$^2H_2$]-3-Hydroxypropanoic Acid *N*-Acetylcysteamine Thiol Ester 2

The above reaction was repeated using deuterated thiol ester **91** (1.80 g, 4.17 mmol) to give *thiol ester* **2** (0.30 g, 38%) as a white solid. δ_H(400 MHz) 2.00 (3H, s, CH_3), 2.40 (1H, br d, *J* 16.5, OH), 2.85 (2H, s, 2-H_2), 3.08 (2H, t, *J* 6.0, CH_2S), 3.49 (2H, app. q, *J* 6.0, CH_2N), 5.87 (1H, br s, NH); δ_D(400 MHz) 3.92 (2D, s, 3-D_2); δ_C(100 MHz) 23.2 (CH_3), 28.8 (CH_2S), 39.2 (CH_2N), 46.4 (C-2), 58.1 (quint., *J* 21.5, C-3), 170.5 (CON), 199.3 (C-1); *m/z* (CI) 194 (MH^+, 97%), 176 (8), 120 (100), 119 (12), 86 (19) and 75 (11). Found (CI): 194.0822 (MH^+), ($C_7H_{12}D_2NO_3S$ requires 194.0820).

1,3-Propanediol 82[174]

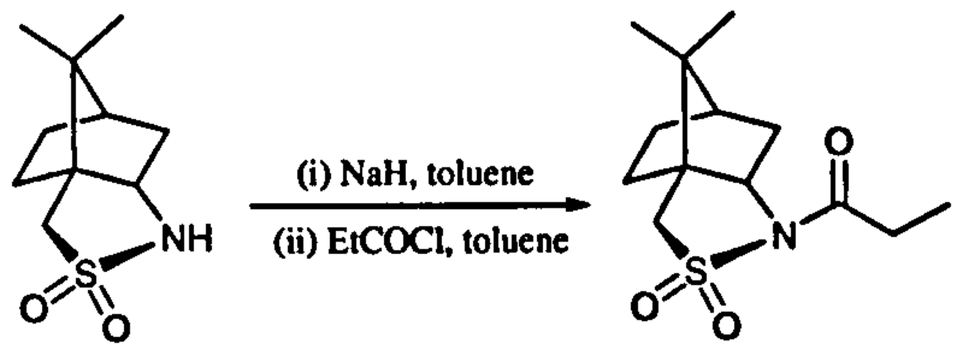
Diethyl malonate **85** (1.00 g, 12.48 mmol) in dry diethyl ether (10 mL) was added dropwise to a slurry of lithium aluminium hydride (2.31 g, 62.4 mmol) in dry diethyl ether (190 mL) under an atmosphere of nitrogen at 0 °C. After 10 minutes, the solution was allowed to warm to room temperature and was stirred overnight. A condenser was attached and then the reaction was quenched using the method of

Leonard *et al.*[175] – water (2.31 mL), aqueous sodium hydroxide solution (15% wt/vol, 2.31 mL) and water (6.93 mL) added dropwise sequentially. Magnesium sulfate was added, the soliuds were allowed to settle and the solution was decanted off and filtered through celite®. The solids remaining in the reaction vessel were washed with THF (2 × 150 mL) and the washings were again decanted off and filtered through celite®. The combined washings were then concentrated *in vacuo* to afford the alcohol **82** (0.24 g, 51%) as a pale yellow oil. δ_H(400 MHz, D_2O) 1.71 (2H, quint., *J* 6.5, 2-H_2), 3.60 (4H, t, *J* 6.5, 1-H_2 and 3-H_2); δ_C(100 MHz, D_2O) 33.8 (C-2), 58.6 (C-1 and C-3).

[1,1,3,3-$^2H_4$]-1,3-Propanediol 87[176]

The above reaction was repeated using diethyl malonate **85** (2.00 g, 24.96 mmol) and lithium aluminium deuteride (2.31 g, 54.91 mmol) to afford the alcohol **87** (0.64 g, 64%) as a colourless oil. δ_H(400 MHz, D_2O) 1.69 (2H, s, 2-H_2); δ_C(100 MHz, D_2O) 33.4 (C-2), 57.9 (quint., *J* 21.5, C-1 and C-3). *m/z* (CI) 81 (MH^+, 26%), 73 (39), 63 (100) and 55 (36).

(1*S*,2*R*)-*N*-Propionyl-bornane-10,2-sultam 104[177,178]

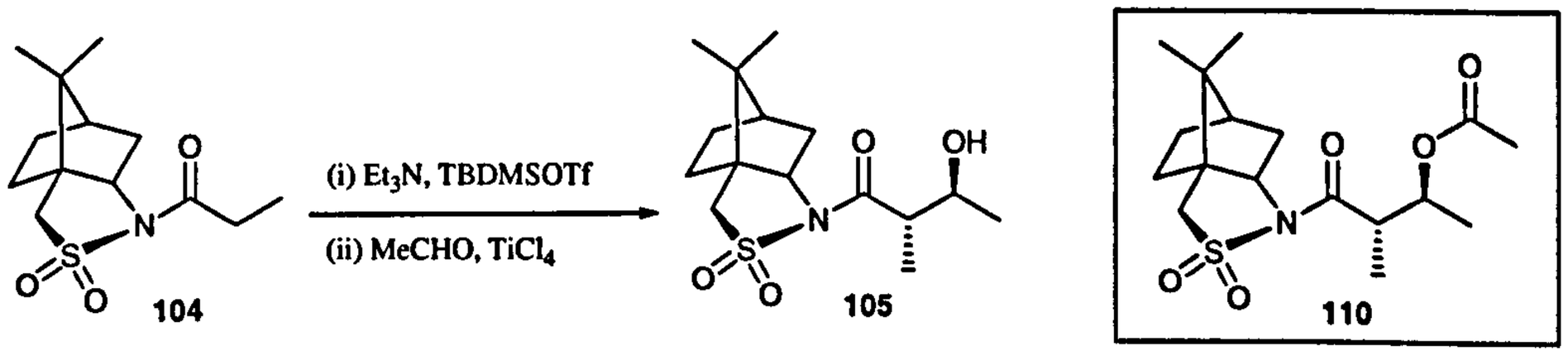


76 104

(1*S*)-(-)-Camphorsultam **76** (2.00 g, 9.33 mmol) in toluene (15 mL) was added dropwise to a stirred suspension of sodium hydride (60% dispersion in oil prewashed with petrol, 0.75 g, 31.7 mmol) in toluene (7 mL) at room temperature for 1 hour. Propionyl chloride (8.10 mL, 93.33 mmol) was added dropwise and the mixture was stirred for 2 hours at room temperature. The reaction was quenched with saturated aqueous ammonium chloride solution (75 mL) and was left to stir for a further 35 minutes. The organic layer was then separated and the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield propionylsultam **104** (2.52 g, 100%) as a white solid. This was then recrystallised from methanol, redissolved in chloroform and evaporated to dryness twice (to remove any residual

methanol) and dried in the oven (due the moisture sensitivity of the next reaction). $[\alpha]_D^{25}$ -116.8 (*c* 2.69, $CHCl_3$); lit.[178] $[\alpha]_D$ -108.4 (*c* 2.65, $CHCl_3$); m.p. 149-152 °C (from ethyl acetate/petroleum ether 40-60 °C), lit.[178] 153-154 °C; δ_H(400 MHz) 0.97 and 1.16 (each 3H, each s, 8-H_3 and 9-H_3), 1.16 (3H, t, *J* 7.5, 3′-H_3), 1.24-1.45 (2H, m, 5-*H*H and 6-*H*H), 1.83-1.97 (3H, m, 4-H, 5-H*H* and 6-H*H*), 2.06 (1H, dd, *J* 14.0, 8.0, 3-*H*H), 2.12 (1H, m, 3-H*H*), 2.72 and 2.80 (each 1H, each dq, *J* 17.0, 7.5, 2′-H_2), 3.43 (1H, d, *J* 14.0, 10-*H*H), 3.50 (1H, d, *J* 14.0, 10-H*H*), 3.87 (1H, dd, *J* 7.5, 5.0, 2-H); δ_C(100 MHz) 8.3 (C-3′), 19.9 and 20.9 (C-8 and C-9), 26.4 and 28.9 (C-5 and C-6), 32.8 (C-3), 38.5 (C-4), 44.6 (C-2′), 47.7 and 48.5 (C-1 and C-7), 52.9 (C-10), 65.2 (C-2), 172.7 (C-1′); *m/z* (CI) 272 (MH^+, 100%), 216 (8), 207 (14), 151 (10), 135 (57), 108 (12), 74 (9) and 57 (19).

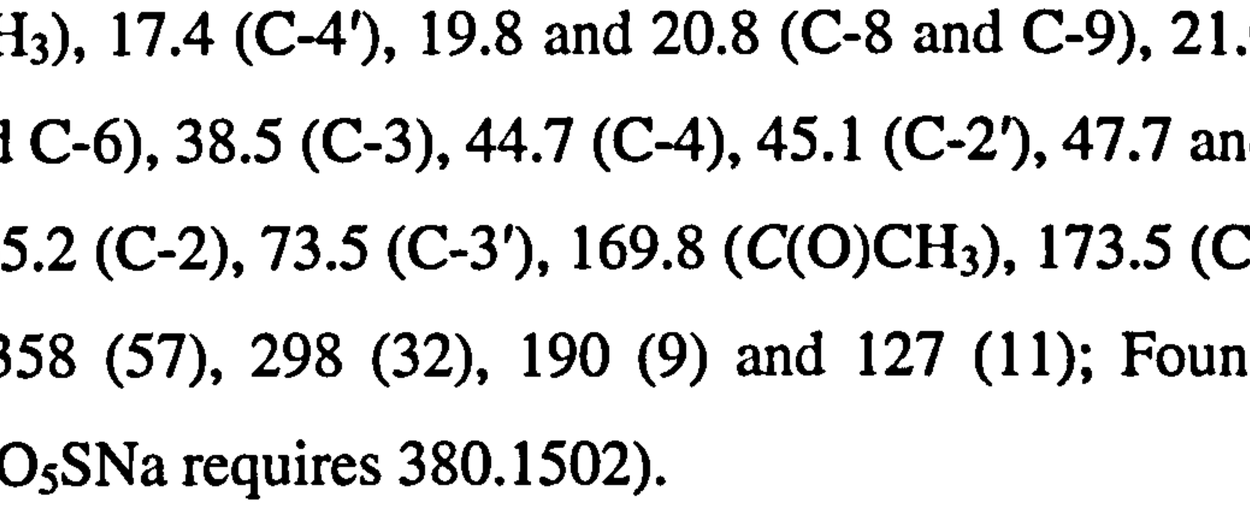
(1*S*,2*R*)-*N*-[(2′*S*,3′*S*)-3-Hydroxy-2-methylbutanoyl]-bornane-10,2-sultam 105[179]



Propionyl sultam **104** (1.00 g, 3.68 mmol) was stirred in dry DCM (7 mL) under an atmosphere of nitrogen at room temperature. Triethylamine (freshly distilled over calcium hydride, 1.5 mL, 11.04 mmol) was added and the reaction was stirred for 5 minutes. *tert*-Butyldimethylsilyltrifluoromethane sulfonate (new bottle, 1.3 mL, 5.52 mmol) was then added and the reaction was stirred for 20 hours. The solution was cooled to -78 ^{0}C and acetaldehyde (0.45 mL, 8.10 mmol) was added. After cooling, titanium (IV) chloride (1.0 M in DCM, 8.1 mL, 8.10 mmol) was added slowly and the solution was stirred for 2 hours. The reaction was then quenched with saturated aqueous ammonium chloride solution (50 mL) and was allowed to warm to room temperature prior to extraction with ethyl acetate (3 × 100 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield an orange-brown oil which solidified upon cooling. Purification by flash chromatography (SiO_2, 30% ethyl acetate/petroleum ether 40-60 °C) gave alcohol **105** as a white solid (1.91 g, 82%). A substantial quantity of *acetate* **110** (40-66%) was observed whenever an excess of acetaldehyde (relative to the quantity of titanium tetrachloride) was used. Alcohol **105**: $[\alpha]_D^{25}$ -72.0 (*c* 1.00, $CHCl_3$), lit.[179] $[\alpha]_D^{20}$ -62.2

(*c* 0.20, $CHCl_3$); m.p. 123-125 °C, lit.[179] 125-126 °C; δ_H(400 MHz) 0.98 and 1.18 (each 3H, each s, 8-H_3 and 9-H_3), 1.21 (3H, d, *J* 7.0, 2′-CH_3), 1.26 (3H, d, *J* 6.5, 4′-H_3), 1.30-1.43 (2H, m, 5-*H*H and 6-*H*H), 1.60 (1H, m, OH), 1.84-1.97 (3H, m, 4-H, 5-H*H* and 6-H*H*), 2.04-2.22 (2H, m, 3-H_2), 3.12 (1H, app. quint., *J* 7.0, 2′-H), 3.46 (1H, d, *J* 14.0, 10-*H*H), 3.54 (1H, d, *J* 14.0, 10-H*H*), 3.84 (1H, m, 3′-H), 3.91 (1H, dd, *J* 7.5, 5.0, 2-H); δ_C(100 MHz) 14.2 (2′-CH_3), 19.9 and 20.8, (C-8 and C-9), 22.0 (C-4′), 26.4 and 33.0 (C-5 and C-6), 38.5 (C-3), 44.7 (C-4), 47.0 (C-2′), 47.8 and 48.3 (C-1 and C-7), 53.2 (C-10), 65.4 (C-2), 71.7 (C-3′), 175.2 (C-1′); *m/z* (CI) 316 (MH^+, 100%), 298 (24), 254 (13), 217 (19), 216 (75), 151 (31) and 135 (34). *Acetate* **110**: $[\alpha]_D^{25}$ -46.8 (*c* 1.93, $CHCl_3$), m.p. 146-148 °C (from $CHCl_3$), ν_{max}(neat)/cm^{-1} 2942, 1735 (C=O), 1686 (C=O), 1455, 1395, 1327, 1234, 1215, 1135, 1065, 944, 776; δ_H(400 MHz) 0.97 (3H, s, 8-H_3 or 9-H_3), 1.17 (3H, d, *J* 7.0, 2′-CH_3), 1.19 (3H, s, 8-H_3 or 9-H_3), 1.27 (3H, d, *J* 6.5, 4′-H_3), 1.31-1.44 (2H, m, 5-*H*H and 6-*H*H), 1.83-1.95 (3H, m, 4-H, 5-H*H* and 6-H*H*), 1.95 (3H, s, C(O)C*H*$_3$), 1.99 (1H, m, 3-*H*H), 2.06 (1H, m, 3-H*H*), 3.28 (1H, dq, *J* 9.0, 7.0, 2′-H), 3.44 (1H, d, 13.5, 10-*H*H), 3.53 (1H, d, *J* 13.5, 10-H*H*), 3.88 (1H, dd, *J* 7.5, 5.0, 2-H), 5.04 (1H, dq, *J* 9.0, 6.5, 3′-H); δ_C(100 MHz) 13.5 (2′-CH_3), 17.4 (C-4′), 19.8 and 20.8 (C-8 and C-9), 21.0 (C(O)*C*H_3), 26.4 and 32.8 (C-5 and C-6), 38.5 (C-3), 44.7 (C-4), 45.1 (C-2′), 47.7 and 48.2 (C-1 and C-7), 53.1 (C-10), 65.2 (C-2), 73.5 (C-3′), 169.8 (*C*(O)CH_3), 173.5 (C-1′); *m/z* (ESI) 380 (MNa^+, 100%), 358 (57), 298 (32), 190 (9) and 127 (11); Found (ESI): 380.1514 (MH^+), ($C_{17}H_{27}NO_5SNa$ requires 380.1502).

(1*S*,2*R*)-*N*-[(2′*S*,3′*S*)-3-(*tert*-Butyldimethylsilanyloxy)-2-methylbutanoyl]-bornane-10,2-sultam 106[88]

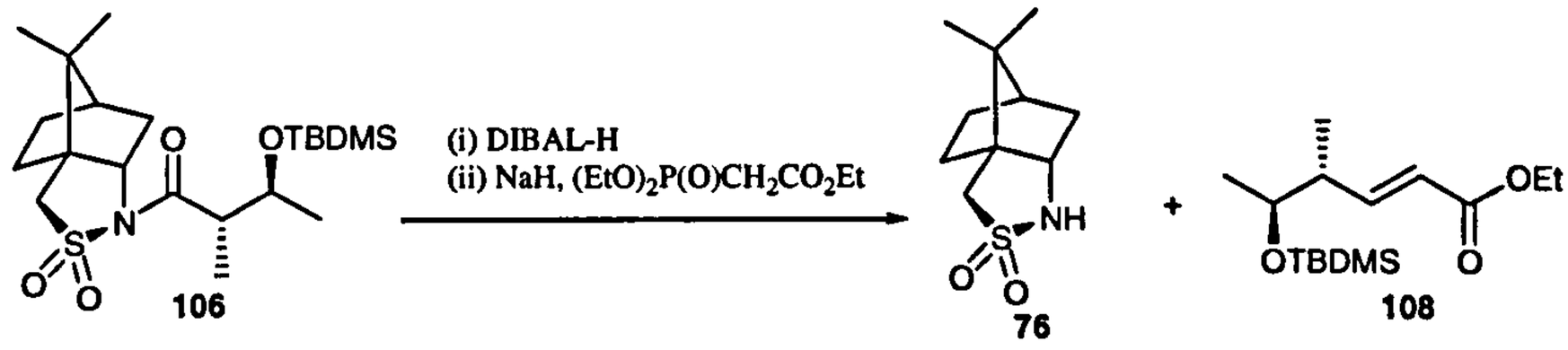


105 106

Alcohol **105** (0.90 g, 2.85 mmol), was dissolved in DCM (10 mL) and cooled to 0 °C. Pyridine (0.5 mL, 0.04 mmol) and *tert*-butyldimethylsilyltrifluoromethane sulfonate (1.0 mL, 4.35 mmol) were added and the mixture was stirred at room temperature for 2 hours. The reaction mixture was quenched with saturated aqueous ammonium chloride solution and left to stir for 35 minutes. The organic layer was removed and

the aqueous layer was extracted with DCM (3 × 50 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo* to furnish silyl ether **106** as a white crystalline solid (1.23 g, 100%). $[\alpha]_D^{22}$-16 (*c* 1.00, $CHCl_3$), lit.[88] $[\alpha]_D$ -14.9 (*c* 0.96, $CHCl_3$); m.p. 143-144 °C (from MeOH), lit.[88] 143-144 °C; δ_H(400 MHz) 0.04 and 0.05 (each 3H, each s, 2 × $SiCH_3$), 0.86 (9H, s, $SiC(CH_3)_3$), 0.96 (3H, s, 8-H_3 or 9-H_3), 1.10 (3H, d, *J* 6.0, 4′-H_3), 1.12 (3H, d, *J* 7.0, 2′-CH_3), 1.16 (3H, s, 8-H_3 or 9-H_3), 1.30-1.43 (2H, m, 5-*H*H and 6-*H*H), 1.83-1.95 (3H, m, 4-H, 5-H*H* and 6-H*H*), 2.04-2.08 (2H, m, 3-H_2), 3.16 (1H, app. quint., *J* 7.0, 2′-H), 3.42 (1H, d, *J* 13.5, 10-*H*H), 3.49 (1H, d, *J* 13.5, 10-H*H*), 3.88 (1H, t, *J* 6.5, 2-H), 4.16 (1H, app. quint., *J* 7.0, 3′-H); δ_C(100 MHz) -4.8 and -4.5 (2 × $SiCH_3$), 11.9 (2′-*C*H_3), 18.0 (Si*C*CH_3), 19.9 and 20.1 (C-8 and C-9), 21.0 (C-4′), 25.8 (3 × $SiCCH_3$), 26.5 and 32.9 (C-5 and C-6), 38.7 (C-3), 44.8 (C-4), 47.7 (C-2′), 48.1 and 48.1 (C-1 and C-7), 53.2 (C-10), 65.5 (C-2), 70.0 (C-3′), 174.5 (C-1′); *m/z* (CI) 430 (M^+, 54%), 414 (68), 372 (100), 330 (66), 298 (32), 215 (46), 159 (33), 115 (32) and 107 (58).

Ethyl (2*E*,4*R*,5*S*)-5-(*tert*-Butyldimethylsilanyloxy)-4-methylhex-2-enoate 108[88]



Silyl ether **106** (1.00 g, 2.33 mmol) was dissolved in dry DCM (10 mL) under an atmosphere of nitrogen and cooled to -78 °C. Diisobutylaluminium hydride (1.0 M in hexanes, 2.3 mL, 2.33 mmol) was added dropwise and the mixture was stirred for 1 hour. The reaction was then quenched with saturated aqueous potassium sodium L-tartrate tetrahydrate solution (6 mL) and was stirred vigorously for 3 hours at room temperature to break up the aluminium complex. The reaction mixture was extracted with DCM (3 × 50 mL) and the combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The resulting oil was triturated with petroleum ether 40-60 °C and filtered to remove the precipitated auxiliary. The filtrate was concentrated *in vacuo* to yield a colourless oil. This was then dissolved in dry THF (3 mL) and added dropwise to a solution of triethyl phosphonoacetate (1.4 mL, 5.70 mmol) and sodium hydride (60% dispersion in oil prewashed with hexane, 0.13 g, 3.22 mmol) in dry THF (45 mL) which had been stirred for 10 minutes at 0 °C

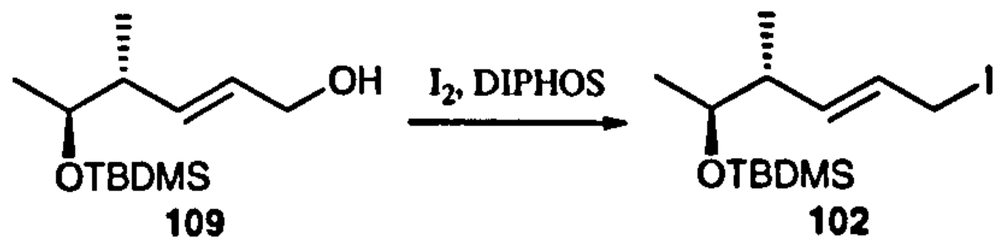
under an atmosphere of nitrogen. The resulting solution was stirred at room temperature for 16 hours and was then quenched with water (35 mL). The solution was extracted with ethyl acetate (3 × 75 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. Purification by flash column chromatography (SiO_2, 2.5% ethyl acetate/petroleum ether 40-60 °C) gave ester **108** (0.55 g, 83%) as a colourless oil. $[\alpha]_D^{22}$ +19.0 (*c* 1.00, $CHCl_3$), lit.[88] $[\alpha]_D$ +18.5 (*c* 0.66, $CHCl_3$); δ_H(400 MHz) 0.04 and 0.05 (each 3H, each s, 2 × $SiCH_3$), 0.88 (9H, s, $SiC(CH_3)_3$), 1.04 (3H, d, *J* 7.0, 4-CH_3), 1.09 (3H, d, *J* 6.0, 6-H_3), 1.29 (3H, t, *J* 7.0, OCH_2CH_3), 2.32 (1H, m, 4-H), 3.74 (1H, dq, *J* 6.0, 5.0, 5-H), 4.19 (2H, q, *J* 7.0, OCH_2CH_3), 5.79 (1H, dd, *J* 16.0, 1.0, 2-H), 6.96 (1H, dd, *J* 16.0, 8.0, 3-H); δ_C(100 MHz) -4.9 and -4.4 (2 × $SiCH_3$), 14.3 (OCH_2CH_3), 15.3 (4-CH_3), 18.1 ($SiC(CH_3)_3$), 21.2 (C-6), 25.8 $C(CH_3)_3$), 44.4 (C-4), 60.1 (OCH_2CH_3), 71.4 (C-5), 121.2 (C-2), 151.6 (C-3), 166.7 (C-1).

(2*E*,4*R*,5*S*)-5-(*tert*-Butyldimethylsilanyloxy)-4-methylhex-2-enol 109[88]

108 **109**

Ethyl ester **108** (0.55 g, 1.90 mmol) was dissolved in dry DCM (20 mL) under an atmosphere of nitrogen at -78 °C. Diisobutylaluminium hydride (1.0 M in hexanes, 4.2 mL, 4.20 mmol) was added dropwise and the solution was stirred at -78 °C for 2 hours. The reaction was then quenched with saturated aqueous potassium sodium L-tartrate tetrahydrate solution (20 mL) and was stirred vigorously at room temperature for 3 hours to break up the aluminium complex. The organic layer was removed and the aqueous layer was extracted with DCM (3 × 50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. Purification by flash column chromatography (SiO_2, 20% ethyl acetate/petroleum ether 40-60 °C) gave alcohol **109** (0.43 g, 92%) as a colourless oil. $[\alpha]_D^{22}$ +12.0 (*c* 1.00, $CHCl_3$), lit.[88] $[\alpha]_D$ +14.7 (*c* 0.86, $CHCl_3$); δ_H(400 MHz) 0.03 and 0.04 (each 3H, each s, 2 × $SiCH_3$), 0.88 (9H, s, $SiC(CH_3)_3$), 0.98 (3H, d, *J* 7.0, 4-CH_3), 1.05 (3H, d, *J* 6.0, 6-H_3), 2.17 (1H, m, 4-H), 3.69 (1H, m, 5-H), 4.09 (2H, s, 1-H_2), 5.63 (2H, m, 2-H and 3-H); δ_C(100 MHz) -4.8 and -4.3 (2 × $SiCH_3$), 16.0 (4-CH_3), 18.1 ($SiC(CH_3)_3$), 20.8 (C-6), 25.9 ($SiC(CH_3)_3$), 44.0 (C-4), 64.0 (C-1), 71.8 (C-5), 129.0 and 135.5 (C-2 and C-3).

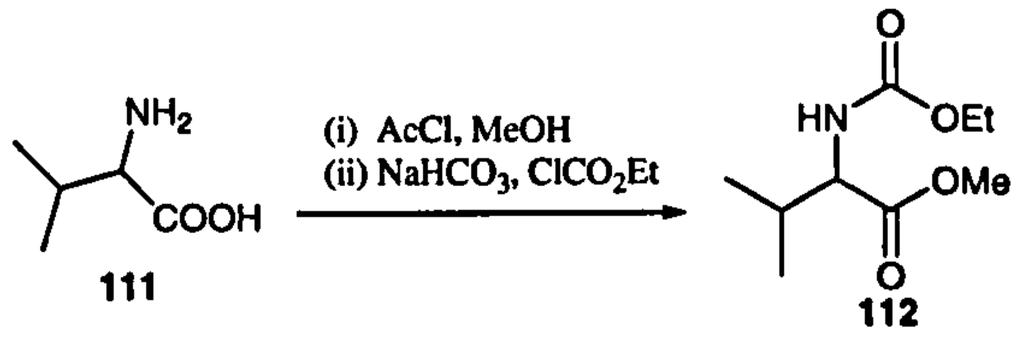
(2*E*, 4*R*, 5*S*)-5-(*tert*-Butyldimethylsilyloxy)-1-iodo-4-methylhex-2-ene 102[88]



109 **102**

Iodine (1.21 g, 4.80 mmol) was added in portions to 1,2-bis(diphenylphosphino)ethane (1.49 g, 3.73 mmol) in dry DCM (4 mL) at 0 °C under an atmosphere of nitrogen and the yellow precipitate which formed was stirred for 10 minutes. Alcohol **109** (0.70 g, 2.86 mmol) in dry DCM (4 mL) was added dropwise and the reaction was stirred for 1 hour. Petrol (25 mL) and diethyl ether (20 mL) were added and the solution was filtered. The solvent was then removed *in vacuo*. Purification by flash column chromatography (SiO_2, 100% petroleum ether 40-60 °C) furnished iodide **102** (0.50 g, 50%) as a pink tinged oil. $[\alpha]_D^{23}$ +10.2 (*c* 1.04, $CHCl_3$); lit.[88] $[\alpha]_D$ +14.8 (*c* 1.11, $CHCl_3$); δ_H(400 MHz) 0.03 and 0.04 (each 3H, each s, each $SiCH_3$), 0.89 (9H, s, $SiC(CH_3)_3$), 0.96 (3H, d, *J* 7.0, 4-CH_3), 1.05 (3H, d, *J* 6.0, 6-H_3), 2.15 (1H, m, 4-H), 3.69 (1H, qd, *J* 6.0, 4.5, 5-H), 3.89 (2H, m, 1-H_2), 5.68-5.72 (2H, m, 2-H and 3-H); δ_C(100 MHz) -4.8 and -4.3 (2 × $SiCH_3$), 7.0 (C-1), 15.8 (4-CH_3), 18.1 ($SiC(CH_3)_3$), 21.0 (C-6), 25.9 ($SiC(CH_3)_3$), 43.8 (C-4), 71.8 (C-5), 127.8 and 137.2 (C-2 and C-3).

Methyl *N*-Ethoxycarbonyl-2-amino-3-methylbutanoate 112[180,181]



111 **112**

Acetyl chloride (21.0 mL, 370.00 mmol) was added dropwise to methanol (100 mL) at 0 °C. D,L-Valine **111** (10.00 g, 85.00 mmol) was added and the mixture was heated to reflux for 4 hours. The mixture was allowed to cool to room temperature and the solvent was removed *in vacuo* to give a colourless oil. Sodium hydrogen carbonate solution (300 mL) was added followed by ethyl chloroformate (10.5 mL, 105.00 mmol) dropwise and then the reaction mixture was stirred overnight. The solution was then extracted with DCM (4 × 100 mL), dried over sodium sulfate, filtered and concentrated in *vacuo* to furnish the ester **112** (15.72 g, 91%) as a colourless oil. This was used without further purification. δ_H(400 MHz) 0.89 and 0.96 (each 3H, each d, *J* 7.0, $CH(CH_3)_2$), 1.25 (3H, t, *J* 7.0, OCH_2CH_3), 2.15 (1H, m, 3-H), 3.74 (3H, s,

OCH_3), 4.12 (2H, q, *J* 7.0, OCH_2CH_3), 4.28 (1H, dd, *J* 9.0, 4.5, 2-H), 5.19 (1H, br d, *J* 9.0, NH); δ_C(100 MHz) 14.5 (OCH_2CH_3), 17.6 ($CHCH_3$), 19.0 ($CHCH_3$), 31.3 (C-3), 52.1 (OCH_3), 58.9 (C-2), 61.2 (CH_2O), 156.5 (CON), 172.7 (C-1).

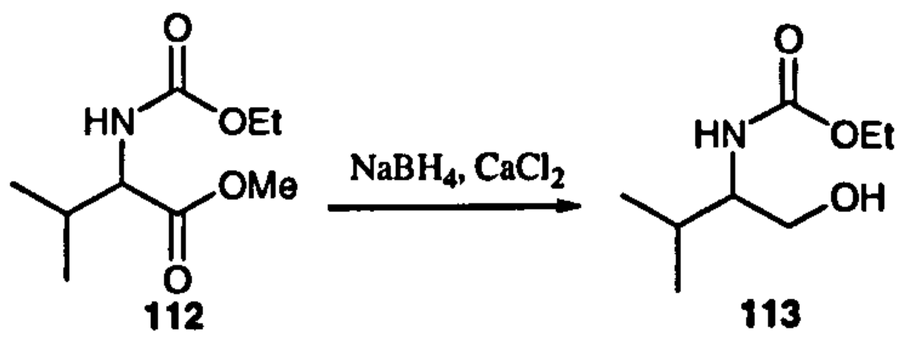
Methyl (*S*)-*N*-Ethoxycarbonyl-2-amino-3-methylbutanoate (*S*)-112[180,181]

The above reaction was repeated using L-valine (5.00 g, 42.50 mmol) to give the desired ester **(*S*)-112** (7.89 g, 91%). $[\alpha]_D^{22}$ +12.07 (*c* 2.32, CH_2Cl_2), lit.[181] $[\alpha]_D^{20}$+5.6 (*c* 1.00, CH_2Cl_2); Spectral data as above.

Methyl (*R*)-*N*-Ethoxycarbonyl-2-amino-3-methylbutanoate (*R*)-112[180,181]

The above reaction was repeated using D-valine (5.00 g, 42.50 mmol) to give the desired ester **(*R*)-112** (7.65 g, 89%). $[\alpha]_D^{22}$ -8.87 (*c* 1.35, CH_2Cl_2), lit.[181] $[\alpha]_D^{20}$+5.6 (*c* 1.00, CH_2Cl_2) for enantiomer; Spectral data as above.

***N*-Ethoxycarbonyl-2-amino-3-methyl-1-butanol 113**[182,183]



112 **113**

Calcium chloride (18.93 g, 170.58 mmol) and sodium borohydride (13.93 g, 368.44 mmol) were added gradually to a solution of ester **112** (15.7 g, 68.23 mmol) in ethanol (160 mL) and THF (45 mL) at 0 °C. The suspension was stirred overnight at room temperature and was then quenched with aqueous citric acid solution (1.0 M, 50 mL). Water (700 mL) was added and the suspension was extracted with ethyl acetate (3 × 400 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to give alcohol **113** (12.28 g, 91%) as a colourless oil. Note: spectral analysis showed that some cyclisation had occurred. δ_H(400 MHz) 0.94 and 0.96 (each 3H, each d, *J* 7.0, $CH(CH_3)_2$), 1.25 (3H, t, *J* 7.0, OCH_2CH_3), 1.85 (1H, m, 3-H), 2.56 (1H, br s, OH), 3.48 (1H, m, 2-H), 3.57-3.76 (2H, m, 1-H_2), 4.12 (2H, q, *J* 7.0, OCH_2CH_3), 4.88 (1H, br d, *J* 8.0, NH); δ_C(100 MHz) 14.6 (CH_3CH_2O), 18.5 ($CHCH_3$), 19.5 ($CHCH_3$), 29.2 (C-3), 58.4 (C-2), 61.0 (CH_3CH_2O), 64.1 (C-1), 157.5 (CO).

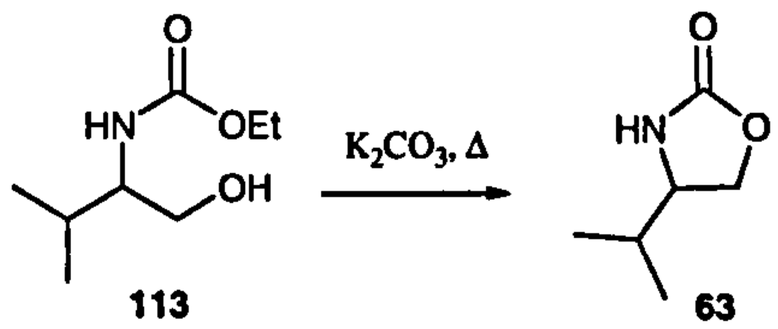
(*S*)-*N*-Ethoxycarbonyl-2-amino-3-methyl-1-butanol (*S*)-113[182,183]

The above reaction was repeated using the ester **(*S*)-112** (7.89 g, 34.29 mmol) to give the desired alcohol **(*S*)-113** (6.39 g, 94%). Note: Spectral analysis showed that some cyclisation had occurred and so $[\alpha]_D$ value has not been included. Spectral data as above.

(*R*)-*N*-Ethoxycarbonyl-2-amino-3-methyl-1-butanol (*R*)-113[182,183]

The above reaction was repeated using the ester **(*R*)-112** (7.65 g, 33.25 mmol) to give the desired alcohol **(*R*)-113** (6.39 g, 97%). Note: Spectral analysis showed that some cyclisation had occurred and so $[\alpha]_D$ value has not been included. Spectral data as above.

4-Isopropyl-2-oxazolidinone (Evans' Auxiliary) 63[184]



113 **63**

Potassium carbonate (0.28 g, 2.10 mmol) was added to a stirred solution of alcohol **113** (12.26 g, 70.0 mmol) in toluene (130 mL). The mixture was heated to reflux overnight using Dean-Stark apparatus. After cooling, the solution was filtered through celite® and washed with ethyl acetate (50 mL). The solution was then dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield oxazolidinone **63** (9.04 g, 100 %) as an opaque oil which solidified to a white solid upon cooling. This was used without further purification. m.p. 70-71 °C (from $CHCl_3$), lit.[184] 69-70 °C; δ_H(400 MHz) 0.91 and 0.97 (each 3H, each d, *J* 6.5, $CH(CH_3)_2$), 1.73 (1H, app. oct., *J* 7.0, $CH(CH_3)_2$), 3.62 (1H, m, 4-H), 4.11 (1H, dd, *J* 9.0, 6.5, 5-*H*H), 4.45 (1H, app. t, *J* 9.0, 5-H*H*), 6.67 (1H, br s, NH); δ_C(100 MHz) 17.6 (CH_3), 18.0 (CH_3), 32.7 ($CH(CH_3)_2$), 58.4 (C-4), 68.6 (C-5), 160.4 (C-2).

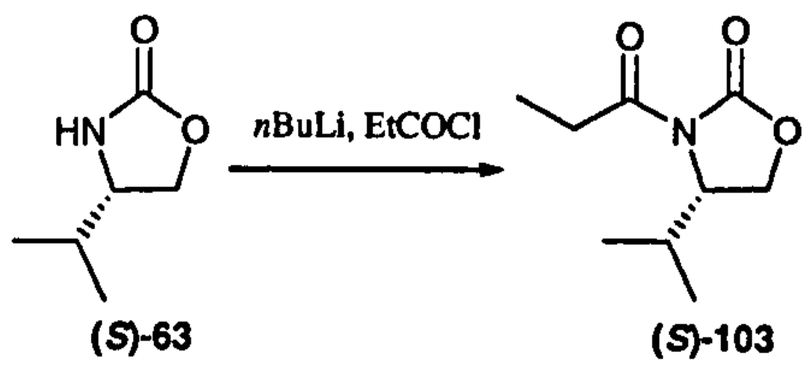
(4*S*)-4-Isopropyl-2-oxazolidinone (Evans' Auxiliary) (*S*)-63[184]

The above reaction was repeated using the alcohol **(*S*)-113** (3.07 g, 23.70 mmol) to give the desired oxazolidinone **(*S*)-63** (2.26 g, 100%). $[\alpha]_D^{23}$ -14.5 (*c* 5.04, EtOH), lit.[184] $[\alpha]_D^{18}$ -16.6 (*c* 5.81, EtOH); Spectral data as above.

(4*R*)-4-Isopropyl-2-oxazolidinone (Evans' Auxiliary) (*R*)-63[184]

The above reaction was repeated using the alcohol **(*R*)-113** (6.35 g, 49.02 mmol) to give the desired oxazolidinone **(*R*)-63** (4.68 g, 100%). $[\alpha]_D^{23}$ +18.9 (*c* 5.51, EtOH), lit.[184] $[\alpha]_D^{18}$ -16.6 (*c* 5.81, EtOH) for enantiomer; Spectral data as above.

(*S*)- 4-Isopropyl-*N*-propyl-2-oxazolidinone (*S*)-103[185]

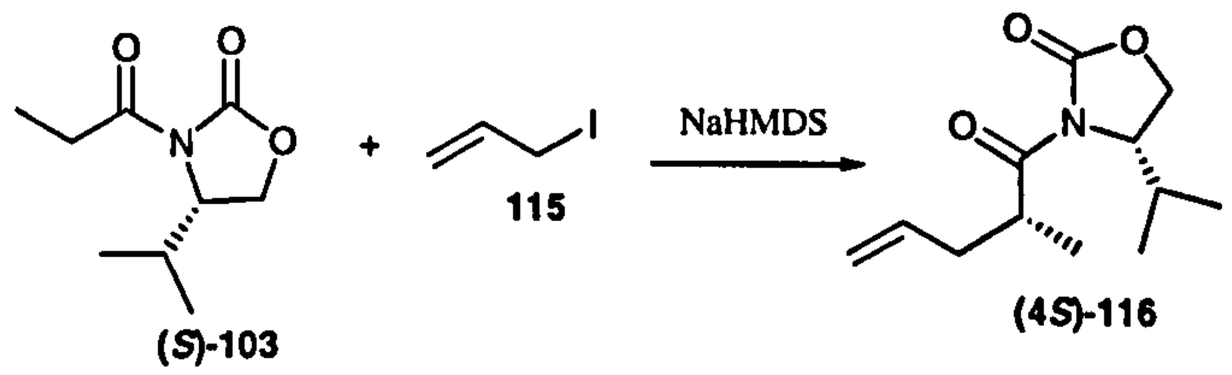


A solution of oxazolidinone **(*S*)-63** (1.91 g, 14.84 mmol) in dry THF (60 mL) was cooled to -78 °C under an atmosphere of nitrogen and treated with *n*-butyllithium (2.5 M in hexanes, 6.6 mL, 16.30 mmol). After 30 minutes, propionyl chloride (1.80 mL, 20.80 mmol) was added dropwise. The mixture was allowed to warm to room temperature overnight and was then quenched with water (20 mL). The mixture was extracted with ethyl acetate (3 × 50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to afford a yellow/orange oil. Purification by flash column chromatography (SiO_2, 20% ethyl acetate/petroleum ether 40-60 °C) furnished the acylated oxazolidinone **(*S*)-103** (2.55 g, 93%) as a pale yellow oil. $[\alpha]_D^{23}$ +95.4 (*c* 8.80, CH_2Cl_2), lit.[185] $[\alpha]_D$ +96.8 (*c* 8.7, CH_2Cl_2); δ_H(400 MHz) 0.88 and 0.92 (each 3H, each d, *J* 7.0, $CH(CH_3)_2$), 1.17 (3H, t, *J* 7.5, 3′-H_3), 2.38 (1H, dsept., *J* 7.0, 4.0, $CH(CH_3)_2$), 2.91 and 2.99 (each 1H, each dq, *J* 17.5, 7.5, 2′-H_2), 4.22 (1H, dd, *J* 9.0, 3.0, 5-*H*H), 4.28 (1H, dd, *J* 9.0, 8.0, 5-H*H*), 4.44 (1H, ddd, *J* 8.0, 4.0, 3.0, 4-H); δ_C(100 MHz) 8.4 (C-3′), 14.6 ($CHCH_3$), 17.9 ($CHCH_3$), 28.3 ($CH(CH_3)_2$), 29.1 (C-2′), 58.4 (C-4), 63.4 (C-5), 154.1 (C-2), 174.0 (C-1′).

(*R*)- 4-Isopropyl-*N*-propyl-2-oxazolidinone (*R*)-103[185]

The above reaction was repeated using **(*R*)-63** (4.00 g, 31.08 mmol) to furnish **(*R*)-103** (4.93 g, 86%) as a pale yellow oil. $[\alpha]_D^{23}$ -81.1 (*c* 9.10, CH_2Cl_2), lit.[185] $[\alpha]_D$ +96.8 (*c* 8.70, CH_2Cl_2) for enantiomer; Spectral data as above.

(4*S*)-4-Isopropyl-*N*-((2′*R*)-2′-methylpent-4′-enoyl)-2-oxazolidinone (4*S*)-116[90,91]

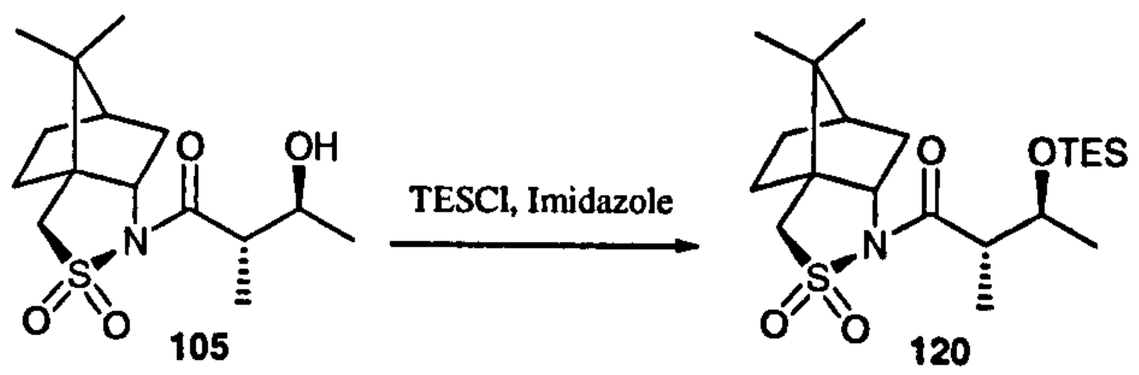


A solution of oxazolidinone **(*S*)-103** (0.40 g, 2.16 mmol) in dry THF (2 mL) was added dropwise to a stirred solution of sodium bis(trimethylsilyl)amide (1.0 M in THF, 2.40 mL, 2.40 mmol) in dry THF (18 mL) at -78 °C under an atmosphere of nitrogen. After 1 hour, allyl iodide **115** (2.16 mL, 23.76 mmol) was added dropwise. The reaction was stirred at -78 °C for 3.5 hours and was then quenched with saturated aqueous ammonium chloride solution (30 mL) and allowed to warm to room temperature. The solution was extracted with DCM (3 × 50 mL). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield a yellow oil. Purification by flash column chromatography (SiO_2, 10% ethyl acetate/petroleum ether 40-60 °C) gave allylic oxazolidinone **(4*S*)-116** (0.34 g, 70%) as a colourless oil. $[\alpha]_D^{24}$ +58.4 (*c* 3.41, CH_2Cl_2); lit.[90] $[\alpha]_D$ +62.9 (*c* 3.48, CH_2Cl_2); δ_H(400 MHz) 0.87 and 0.91 (each 3H, each d, *J* 7.0, $CH(CH_3)_2$), 1.15 (3H, d, *J* 7.0, 2′-CH_3), 2.20 (1H, app. dtt, *J* 14.0, 7.0, 1.0, 3′-*H*H), 2.32 (1H, dsept., *J* 7.0, 4.0, C*H*$(CH_3)_2$), 2.51 (1H, app. dtt, *J* 14.0, 7.0, 1.0, 3′-H*H*), 3.89 (1H, app. sext., *J* 7.0, 2′-H), 4.20 (1H, dd, *J* 9.0, 3.0, 5-*H*H), 4.27 (1H, dd, *J* 9.0, 8.5, 5-H*H*), 4.46 (1H, ddd, *J* 8.5, 3.5, 3.0, 4-H), 5.01-5.11 (2H, m, 5′-H_2), 5.79 (1H, ddt, *J* 17.0, 10.0, 7.0, 4′-H); δ_C(100 MHz) 14.6 ($CHCH_3$), 16.2 (2′-CH_3), 18.0 ($CHCH_3$), 28.4 ($CH(CH_3)_2$), 37.1 (C-2′), 38.2 (C-3′), 58.4 (C-4), 63.1 (C-5), 117.1 (C-5′), 135.2 (C-4′), 153.72 (C-2), 176.4 (C-1′).

(4*R*)-4-Isopropyl-*N*-((2′*S*)-2′-methylpent-4′-enoyl)- 2-oxazolidinone (4*R*)-116[90,91]

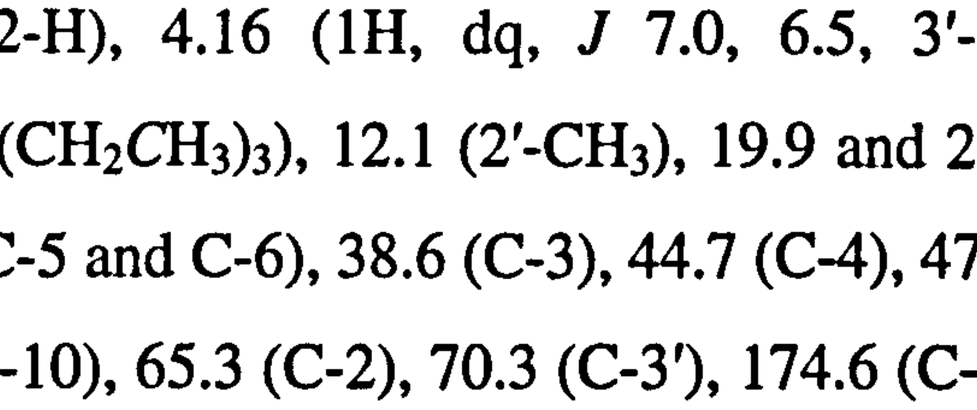
The above reaction was repeated using **(*R*)-103** (2.00 g, 10.80 mmol) to furnish **(4*R*)-116** (1.75 g, 72%) as a pale yellow oil. $[\alpha]_D^{23}$ - 56.7 (*c* 3.56, CH_2Cl_2); lit.[90] $[\alpha]_D$ +62.9 (*c* 3.48, CH_2Cl_2) for enantiomer. Spectral data as above.

(1*S*,2*R*)-*N*-[(2′*S*,3′*S*)-3-(Triethylsilanyloxy)-2-methylbutanoyl]-bornane-10,2-sultam 120



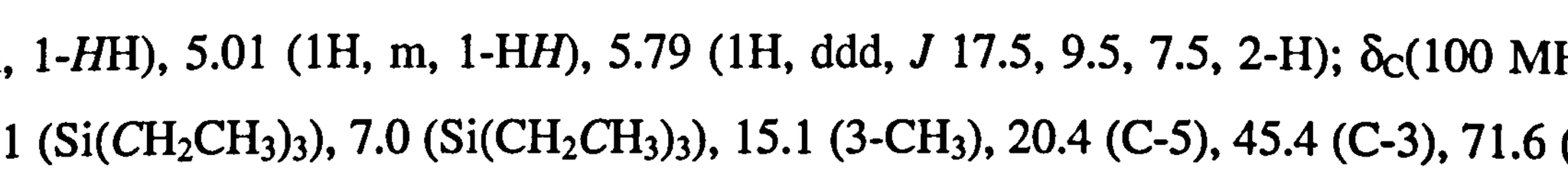
Alcohol **105** (4.47 g, 14.17 mmol) was dissolved in dry DMF (17 mL) under an atmosphere of nitrogen. Imidazole (1.26 g, 18.42 mmol) was added and the reaction was cooled to 0 °C. Chlorotriethylsilane (2.85 mL, 17.00 mmol) was added and the mixture was stirred for 19 hours at room temperature. The solution was then diluted with diethyl ether (50 mL) causing a white precipitate and washed with water (3 × 20 mL). The organic layer was then dried over magnesium sulfate, filtered and concentrated *in vacuo*. Purification by flash column chromatography (SiO_2, 5-10% ethyl acetate/petroleum ether 40-60 °C) gave *silyl ether* **120** (5.28 g, 87%) as a white solid. Found: C, 58.60; H, 9.07; N, 3.36; $C_{21}H_{39}NO_4SSi$ requires C, 58.70; H, 9.15; N, 3.26; $[\alpha]_D^{22}$ -13.8 (*c* 5.35, $CHCl_3$); m.p. 148-150 °C (from ethyl acetate and petroleum ether 40-60 °C); ν_{max}(neat)/cm^{-1} 2955, 2877, 1685 (C=O), 1458, 1333, 1213, 1132, 1108, 1012, 971, 943, 770, 723; δ_H(400 MHz) 0.58 (6H, q, *J* 8.0, $Si(CH_2CH_3)_3$), 0.93 (9H, t, *J* 8.0, $Si(CH_2CH_3)_3$), 0.97 (3H, s, 8-H_3 or 9-H_3), 1.11 (3H, d, *J* 7.0, 2′-CH_3), 1.14 (3H, d, *J* 6.5, 4′-H_3), 1.18 (3H, s, 8-H_3 or 9-H_3), 1.26-1.44 (2H, m, 5-*H*H and 6-*H*H), 1.83-1.96 (3H, m, 4-H, 5-H*H* and 6-H*H*), 2.03-2.08 (2H, m, 3-H_2), 3.14 (1H, app. quint., *J* 7.0, 2′-H), 3.42 (1H, d, *J* 13.5, 10-*H*H), 3.50 (1H, d, *J* 13.5, 10-H*H*), 3.88 (1H, t, *J* 6.5, 2-H), 4.16 (1H, dq, *J* 7.0, 6.5, 3′-H); δ_C(100 MHz) 4.9 ($Si(CH_2CH_3)_3$), 6.8 ($Si(CH_2CH_3)_3$), 12.1 (2′-CH_3), 19.9 and 20.2 (C-8 and C-9), 21.0 (C-4′), 26.5 and 32.8 (C-5 and C-6), 38.6 (C-3), 44.7 (C-4), 47.7 and 48.1 (C-1 and C-7), 48.2 (C-2′), 53.1 (C-10), 65.3 (C-2), 70.3 (C-3′), 174.6 (C-1′); *m/z* (CI) 430 (MH^+, 19%), 400 (100), 330 (65), 159 (12), 135 (20) and 115 (22); Found (CI): 430.2439 (MH^+), ($C_{21}H_{40}NO_4SSi$ requires 430.2447).

(3*R*,4*S*)-3-Methyl-4-triethylsilanyloxypent-1-ene 117[62]



Diisobutylaluminium hydride (1.0 M in hexanes, 5.0 mL, 5.00 mmol) was added dropwise over 30 minutes to silyl ether **120** (2.00 g, 4.84 mmol) in dry DCM (24 mL) under an atmosphere of nitrogen at -78 °C. The reaction was stirred for 5 hours and then quenched with saturated aqueous potassium sodium L-tartrate tetrahydrate solution (40 mL). The solution was stirred vigorously overnight and was then extracted with DCM (3 × 100 mL), dried over magnesium sulfate and concentrated *in vacuo*. The resulting solid was triturated with petroleum ether 40-60 °C and filtered to remove the precipitated auxiliary. The filtrate was concentrated *in vacuo* to yield a pale yellow oil. Meanwhile, a solution of methyltriphenylphosphonium bromide (8.74 g, 24.20 mmol) and sodium hydride (60% dispersion in oil prewashed with dry hexane, 4.36 g, 109.20 mmol,) in dry THF (100 mL) under an atmosphere of nitrogen was gently heated with stirring until a green/grey colour was observed. The solution was stirred overnight at room temperature and the solids were then allowed to settle. The bright yellow solution was transferred *via* cannula into a solution of the aldehyde in dry THF (3 mL) and left to stir at room temperature under an atmosphere of nitrogen for 3 hours. The solvent was then removed *in vacuo* and the resulting yellow residue was purified by flash column chromatography (SiO_2, 0-1% ethyl acetate/petroleum ether 40-60 °C) to give alkene **117** (0.55 g, 55%) as a colourless oil. $[\alpha]_D^{21}$ +10.5 (*c* 2.48, $CHCl_3$); lit.[62] $[\alpha]_D$ +2.8 (*c* 2.50, $CHCl_3$); δ_H(400 MHz) 0.59 (6H, q, *J* 8.0, $Si(CH_2CH_3)_3$), 0.96 (9H, t, *J* 8.0, $Si(CH_2CH_3)_3$), 0.99 (3H, d, *J* 7.0, 3-CH_3), 1.07 (3H, d, *J* 6.5, 5-H_3), 2.18 (1H, m, 3-H), 3.73 (1H, qd, *J* 6.5, 4.5, 4-H), 4.98 (1H, m, 1-*H*H), 5.01 (1H, m, 1-H*H*), 5.79 (1H, ddd, *J* 17.5, 9.5, 7.5, 2-H); δ_C(100 MHz) 5.1 ($Si(CH_2CH_3)_3$), 7.0 ($Si(CH_2CH_3)_3$), 15.1 (3-CH_3), 20.4 (C-5), 45.4 (C-3), 71.6 (C-4), 114.2 (C-2), 141.3 (C-1).

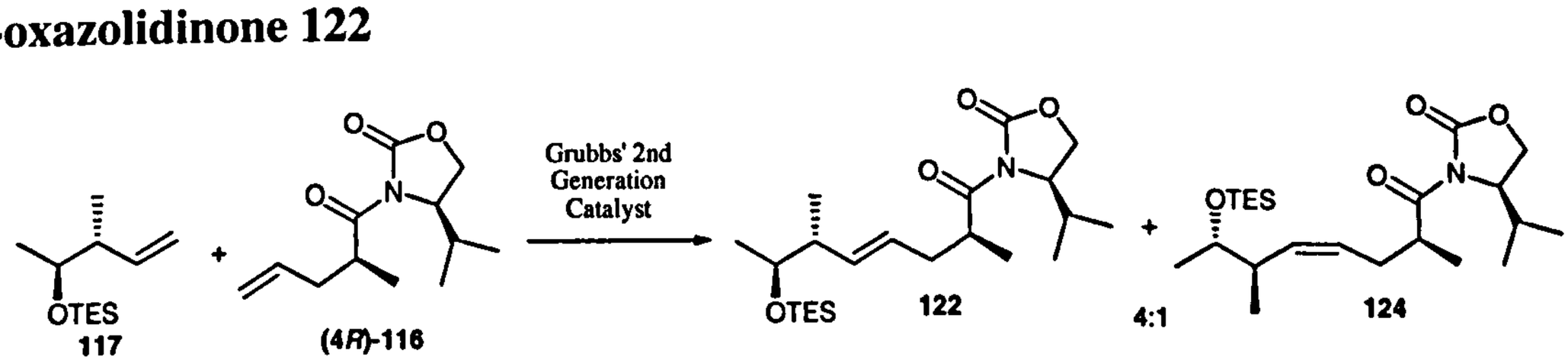
(4*S*)-*N*-((2*R*,4*E*,6*R*,7*S*)-2,6-Dimethyl-7-triethylsilanyloxyoct-4-enoyl)-4-isopropyl-2-oxazolidinone 121



To a solution of Grubbs' 2nd generation catalyst (24.3 mg, 0.028 mmol) in dry toluene (2 mL) was added a premixed solution of alkene **117** (0.24 g, 1.12 mmol) and alkene **(4*S*)-116** (0.12 g, 0.56 mmol) in dry toluene (3 mL). The reaction was stirred

at 55-65 °C overnight. Upon cooling, the solvent was removed *in vacuo* and the mixture was then purified by flash column chromatography (SiO_2, 2.5-5% ethyl acetate/petroleum ether 40-60 °C) to give an inseparable 4:1 mixture of *alkenes* **121** and **123** (0.12 g, 50%) as a colourless oil. $[\alpha]_D^{25}$ (of 4:1 mixture) +14.2 (*c* 3.32, $CHCl_3$); ν_{max}(neat)/cm^{-1} 2961, 2876, 1779 (C=O), 1700 (C=O), 1384, 1372, 1235, 1201, 1093, 1006, 956; δ_H(400 MHz) (major isomer) 0.58 (6H, q, *J* 8.0, $Si(CH_2CH_3)_3$), 0.87 and 0.91 (each 3H, each d, *J* 7.0, $CH(CH_3)_2$), 0.94 (3H, d, *J* 6.0, 6′-CH_3), 0.95 (9H, t, *J* 8.0, $Si(CH_2CH_3)_3$), 1.03 (3H, d, *J* 6.0, 8′-H_3), 1.13 (3H, d, *J* 7.0, 2′-CH_3), 2.08-2.19 (2H, m, *J* 7.0, 3′-*H*H and 6′-H), 2.33 (1H, dsept., *J* 7.0, 3.5, $CH(CH_3)_2$), 2.47 (1H, dt, *J* 13.5, 7.0, 3′-H*H*), 3.70 (1H, qd, *J* 6.0, 4.5, 7′-H), 3.80 (1H, app. sext., *J* 7.0, 2′-H), 4.20 (1H, dd, *J* 9.0, 3.0, 5-*H*H), 4.27 (1H, dd, *J* 9.0, 8.5, 5-H*H*), 4.46 (1H, ddd, *J* 8.5, 3.5, 3.0, 4-H), 5.35 (1H, dt, *J* 15.5, 7.0, 4′-H), 5.45 (1H, dd, *J* 15.5, 7.5, 5′-H); δ_C(100 MHz) (major isomer) 5.0 ($Si(CH_2CH_3)_3$), 6.9 ($Si(CH_2CH_3)_3$), 14.7 ($CH(CH_3)_2$), 15.3 and 16.0 (2′-CH_3 and 6′-CH_3), 18.0 ($CH(CH_3)_2$), 20.2 (C-8′), 28.4 ($CH(CH_3)_2$), 37.1 (C-3′), 37.7 (C-2′), 44.2 (C-6′), 58.4 (C-4), 63.1 (C-5), 71.7 (C-7′), 126.4 (C-4′), 135.7 (C-5′), 153.7 (C-2), 176.6 (C-1′); *m/z* (CI) 412 (MH^+, 3%), 382 (60), 280 (100), 159 (28) and 151 (23); Found (CI): 412.2885 (MH^+), ($C_{22}H_{42}NO_4Si$ requires 412.2883).

(4*R*)-*N*-((2*S*,4*E*,6*R*,7*S*)-2,6-Dimethyl-7-triethylsilanyloxyoct-4-enoyl)-4-isopropyl-2-oxazolidinone 122

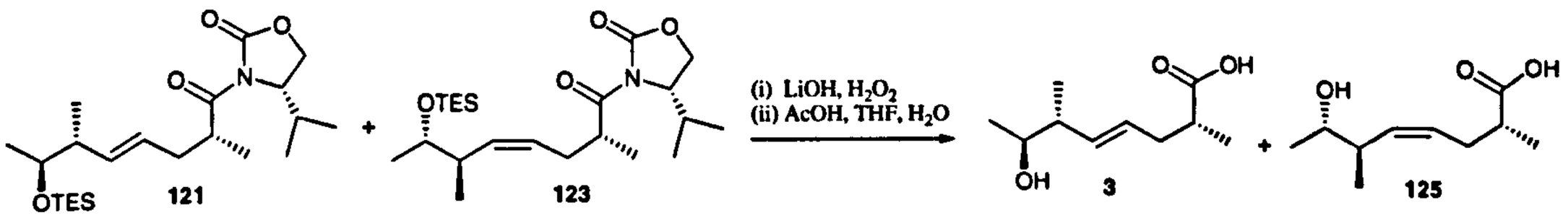


To a solution of Grubbs' 2nd generation catalyst (60.8 mg, 0.07 mmol) in dry toluene (5 mL) was added a premixed solution of and alkene **117** (0.60 g, 2.80 mmol) and alkene **(4*R*)-116** (0.31 g, 1.40 mmol) in dry toluene (7 mL). The reaction was stirred at 60-70 °C overnight. Upon cooling, the solvent was removed *in vacuo* and the mixture was then purified by flash column chromatography (SiO_2, 2.5-5% ethyl acetate/petroleum ether 40-60 °C) to give an inseparable 4:1 mixture of *alkenes* **122** and **124** (0.22 g, 38%) as a colourless oil. $[\alpha]_D^{25}$ (of 4:1 mixture) -22.7 (*c* 2.56, $CHCl_3$); ν_{max}(neat)/cm^{-1} 2960, 2876, 1779 (C=O), 1700 (C=O), 1458, 1384, 1372,

1235, 1200, 1093, 1006, 964, 740, 724; δ_H(400 MHz) (major isomer) 0.58 (6H, q, *J* 8.0, Si(C*H*$_2$CH$_3$)$_3$), 0.87 and 0.91 (each 3H, each d, *J* 7.0, CH(C*H*$_3$)$_2$), 0.94 (3H, d, *J* 6.5, 6′-CH$_3$), 0.95 (9H, t, *J* 8.0, Si(CH$_2$C*H*$_3$)$_3$), 1.03 (3H, d, *J* 6.0, 8′-H$_3$), 1.13 (3H, d, *J* 7.0, 2′-CH$_3$), 2.08-2.20 (2H, m, 3′-*H*H and 6′-H), 2.33 (1H, dsept., *J* 7.0, 3.5, C*H*(CH$_3$)$_2$), 2.47 (1H, app. dt, *J* 14.0, 6.0, 3′-H*H*), 3.70 (1H, qd, *J* 6.0, 4.5, 7′-H), 3.80 (1H, app. sext., *J* 7.0, 2′-H), 4.20 (1H, dd, *J* 9.0, 3.0, 5-*H*H), 4.27 (1H, dd, *J* 9.0, 8.5, 5-H*H*), 4.46 (1H, ddd, *J* 8.5, 3.5, 3.0, 4-H), 5.35 (1H, m, 4′-H), 5.45 (1H, dd, *J* 15.5, 7.0, 5′-H); δ_C(100 MHz) (major isomer) 4.9 (Si(*C*H$_2$CH$_3$)$_3$), 6.8 (Si(CH$_2$*C*H$_3$)$_3$), 14.6 (CH(*C*H$_3$)$_2$), 15.0 and 15.8 (2′-CH$_3$ and 6′-CH$_3$), 17.9 (CH(*C*H$_3$)$_2$), 20.0 (C-8′), 28.4 (*C*H(CH$_3$)$_2$), 37.0 (C-3′), 37.6 (C-2′), 44.1 (C-6′), 58.3 (C-4), 63.0 (C-5), 71.6 (C-7′), 126.3 (C-4′), 135.6 (C-5′), 153.6 (C-2), 176.4 (C-1′); *m/z* (CI) 412 (MH$^+$, 2%), 382 (70), 280 (100), 159 (48) and 151 (32); Found (CI): 412.2866 (MH$^+$), ($C_{22}H_{42}NO_4Si$ requires 412.2883).

(2*R*,4*E*,6*R*,7*S*)-2,6-Dimethyl-7-hydroxyoct-4-enoic acid (mupiric acid) 3

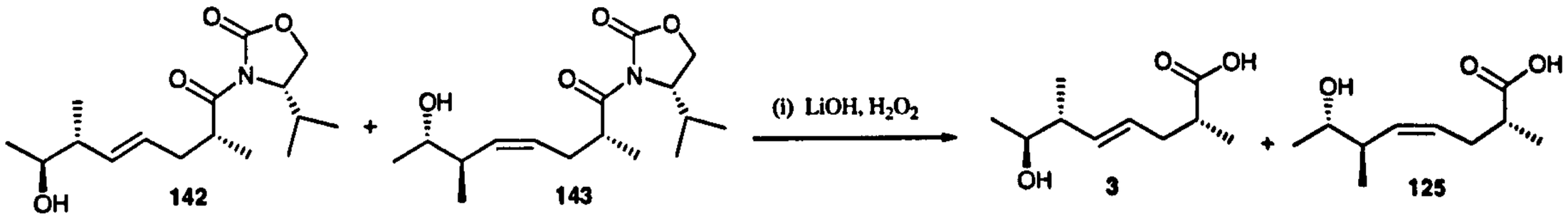
(i) *via* deprotection and auxiliary cleavage of silyl ether 121



Lithium hydroxide monohydrate (0.02 g, 0.50 mmol) and hydrogen peroxide solution (30% solution in water, 0.1 mL, 0.75 mmol) were added successively to a solution of alkenes **121** and **123** (0.11 g, 0.28 mmol) in THF (3 mL) and water (1.5 mL) at 0 °C. This was then stirred at room temperature overnight and quenched with sodium sulfite solution (7.6% in water, 1.3 mL, 0.75 mmol). After 10 minutes the solvents were removed *in vacuo*. A mixture of water (3 mL), acetic acid (5 mL) and THF (11 mL) was then added to the residue and the mixture was stirred overnight. Solvents were removed *in vacuo* and the crude product was purified by flash column chromatography (SiO_2, 20% ethyl acetate/1% acetic acid/petroleum ether 40-60 °C) to afford an inseparable 4:1 mixture of *acids* **3** and **125** (0.20 g, 40%) as a colourless oil. $[\alpha]_D^{25}$ (of 4:1 mixture) +13.4 (*c* 1.05, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3363 (OH), 2971, 2933, 1705 (C=O), 1457, 1378, 1226, 1189, 1086, 971; δ_H(400 MHz) (major isomer) 0.98 (3H, d, *J* 7.0, 6-CH$_3$), 1.16 (3H, d, *J* 6.5, 8-H$_3$), 1.18 (3H, d, *J* 7.0, 2-CH$_3$), 2.09 (1H, m, 6-H), 2.25 (1H, app. dtd, *J* 14.0, 7.0, 1.0, 3-*H*H), 2.35 (1H, app. dt, *J* 14.0,

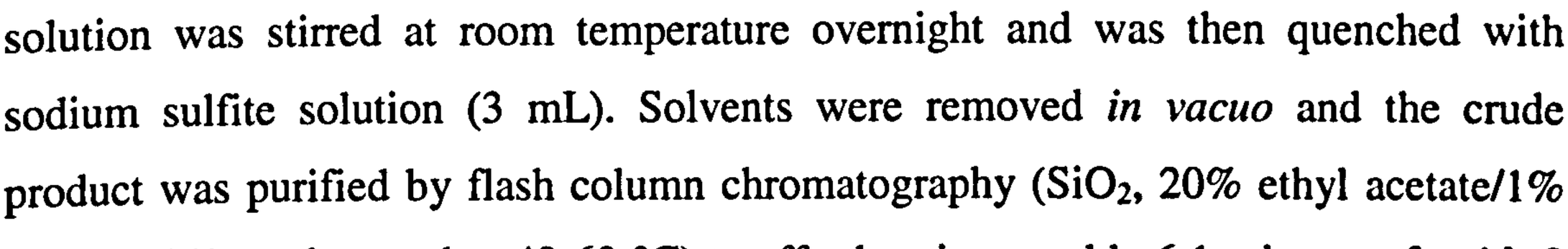
7.0, 1.0, 3-H*H*), 2.56 (1H, app. sext., *J* 7.0, 2-H), 3.53 (1H, app. quint., *J* 6.5, 7-H), 5.39 (1H, dd, *J* 15.5, 8.5, 5-H), 5.53 (1H, dt, *J* 15.5, 7.0, 4-H), 6.50 (1H, br s, OH); δ_H(400 MHz, C_6D_6) (major isomer) 0.90 (3H, d, *J* 7.0, 6-CH_3), 1.04 (3H, d, *J* 7.0, 2-CH_3), 1.12 (3H, d, *J* 6.0, 8-H_3), 1.96 (1H, m, 6-H), 2.11 (1H, app. dtd, *J* 13.5, 7.0, 3-*H*H), 2.22 (1H, app. dtd, *J* 13.5, 7.0, 3-H*H*), 2.41 (1H, m, 2-H), 3.46 (1H, app. quint., *J* 6.0, 7-H), 5.34 (1H, dd, *J* 15.5, 8.0, 5-H), 5.41 (1H, dt, *J* 15.5, 7.0, 4-H), 5.94 (1H, br s, 2 × OH); δ_C(100 MHz) (major isomer) 16.2 and 16.5 (2-CH_3 and 6-CH_3), 20.0 (C-8), 36.5 (C-3), 39.8 (C-2), 44.9 (C-6), 71.1 (C-7), 128.7 (C-4), 134.9 (C-5), 181.2 (C-1); *m/z* (CI) 187 (MH^+, 5%), 169 (63), 151 (78), 123 (67), 95 (100), 85 (28), 83 (36) and 69 (43); Found (CI): 187.1332 (MH^+), ($C_{10}H_{19}O_3$ requires 187.1334).

(ii) *Via* auxiliary cleavage of alcohol 142



Lithium hydroxide monohydrate (0.14 g, 0.36 mmol) and hydrogen peroxide solution (30% in water, 0.07 mL, 0.56 mmol) were added successively to a solution of alcohols **142** and **143** (0.072 g, 0.24 mmol) in THF (3 mL) and water (1.5 mL). The solution was stirred at room temperature overnight and was then quenched with sodium sulfite solution (3 mL). Solvents were removed *in vacuo* and the crude product was purified by flash column chromatography (SiO_2, 20% ethyl acetate/1% acetic acid/petroleum ether 40-60 °C) to afford an inseparable 6:1 mixture of *acids* **3** and **125** (0.012 g, 27%) as a colourless oil. $[\alpha]_D^{25}$ (of 6:1 mixture) +11.5 (*c* 0.61, $CHCl_3$); Spectral data as above.

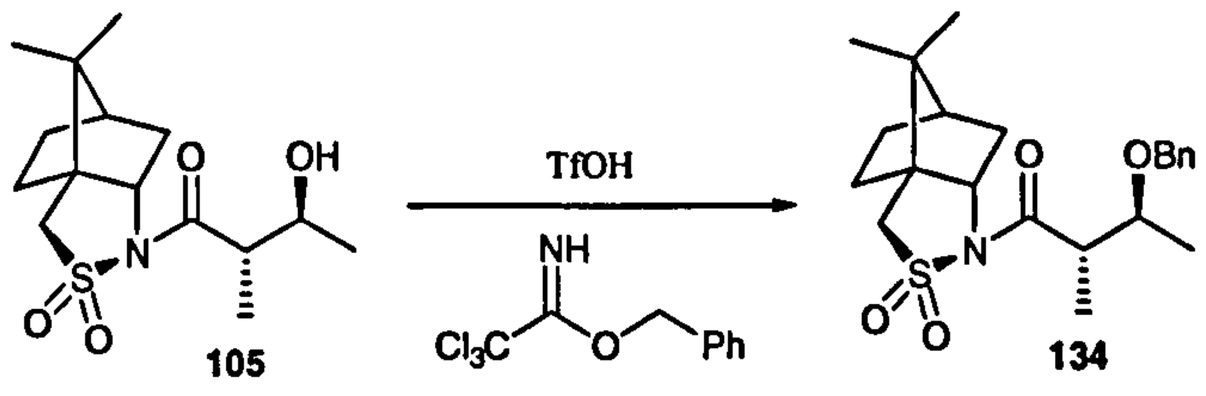
(2*S*, 4*E*, 6*R*, 7*S*)-2,6-Dimethyl-7-hydroxyoct-4-enoic acid 4



Lithium hydroxide monohydrate (0.02 g, 0.50 mmol) and hydrogen peroxide solution (30% solution in water, 0.1 mL, 0.75 mmol) were added successively to a solution of alkenes **122** and **124** (0.13 g, 0.32 mmol) in THF (3 mL) and water (1.5 mL) at 0 °C. This was then stirred at room temperature overnight and quenched with sodium sulfite solution (7.6% in water, 1.25 mL, 0.75 mmol). After 10 minutes the solvents were

removed *in vacuo*. A mixture of water (3 mL), acetic acid (5 mL) and THF (11 mL) was then added to the residue and the mixture was stirred overnight. Solvents were removed *in vacuo* and the crude product was purified by flash column chromatography (SiO_2, 20% ethyl acetate/1% acetic acid/petroleum ether 40-60 °C) to afford an inseparable 4:1 mixture of *acids* **4** and **126** (0.45 g, 76%) as a colourless oil. $[\alpha]_D^{25}$ (of 4:1 mixture) +35.0 (*c* 1.95, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3333 (OH), 2971, 2933, 1705 (C=O), 1456, 1377, 1228, 1191, 1086, 971; δ_H(400 MHz) (major isomer) 0.98 (3H, d, *J* 7.0, 6-CH_3), 1.16 (3H, d, *J* 6.0, 8-H_3), 1.18 (3H, d, *J* 7.0, 2-CH_3), 2.07 (1H, m, 6-H), 2.21 (1H, app. dtd, *J* 14.0, 7.0, 1.0, 3-*H*H), 2.34 (1H, app. dt, *J* 14.0, 7.0, 3-H*H*), 2.52 (1H, m, 2-H), 3.52 (1H, app. quint., *J* 6.0, 7-H), 5.35 (1H, dd, *J* 15.0, 8.5, 5-H), 5.51 (1H, dt, *J* 15.0, 7.0, 4-H), 6.07 (1H, br s, OH); δ_H(400 MHz, C_6D_6) (major isomer) 0.98 (3H, d, *J* 7.0, 6-CH_3), 1.05 (3H, d, *J* 7.0, 2-CH_3), 1.14 (3H, d, *J* 6.0, 8-H_3), 1.92 (1H, m, 6-H), 2.04 (1H, app. dtd, *J* 13.5, 5.5, 1.0, 3-*H*H), 2.26 (1H, app. dt, *J* 13.5, 8.5, 3-H*H*), 2.40 (1H, m, 2-H), 3.47 (1H, app. quint., *J* 6.0, 7-H), 5.31 (1H, dd, *J* 15.5, 8.0, 5-H), 5.39 (1H, dt, *J* 15.5, 7.0, 4-H), 6.32 (1H, br s, 2 × OH); δ_C(100 MHz) (major isomer) 16.6 (2 coincident peaks, 2-CH_3 and 6-CH_3), 19.9 (C-8), 37.0 (C-3), 39.7 (C-2), 45.1 (C-6), 71.2 (C-7), 129.3 (C-4), 134.9 (C-5), 180.8 (C-1); *m/z* (CI) 187 (MH^+, 31%), 169 (100), 151 (93), 123 (82), 95 (98), 85 (10), 83 (16), 74 (23) and 69 (49); Found (CI): 187.1334 (MH^+), ($C_{10}H_{19}O_3$ requires 187.1334).

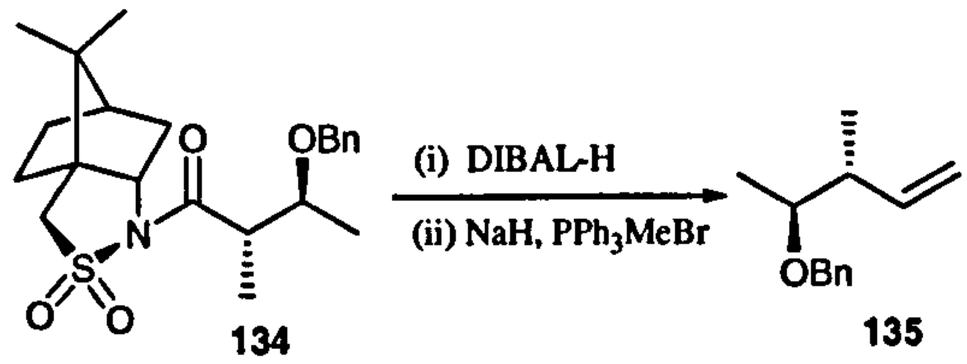
(1*S*,2*R*)-*N*-[(2′*S*,3′*S*)-3-Benzyloxy-2-methylbutanoyl]-bornane-10,2-sultam 134



Trifluoromethanesulfonic acid (0.4 mL, 3.96 mmol) was added to a stirred solution of alcohol **105** (8.30 g, 26.33 mmol) and benzyl 2,2,2-trichloroacetimidate (6.4 mL, 33.90 mmol) in a mixture of cyclohexane (40 mL) and dry DCM (40 mL) at room temperature under an atmosphere of nitrogen. The solution was stirred for 3 hours and then filtered through celite to remove the crystalline trichloroacetimidate. The solid was washed with DCM and the combined organic extracts were adjusted to pH 7 with saturated aqueous sodium hydrogen carbonate solution. The extracts were then washed with saturated sodium hydrogen carbonate solution (40 mL) and water (40

mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield a white solid. Purification by flash column chromatography (SiO_2, 0-30% ethyl acetate/petroleum ether 40-60 °C) gave *benzyl ether* **134** (10.55 g, 99%) as a white crystalline solid. Found: C, 65.01; H, 7.91; N, 3.31; S, 7.68; $C_{22}H_{31}NO_4S$ requires C, 65.15; H, 7.70; N, 3.45; S, 7.91; $[\alpha]_D^{21}$ +23.5 (*c* 1.02, $CHCl_3$); m.p. 148-150 °C (from ethyl acetate and petroleum ether 40-60 °C); ν_{max}(neat)/cm^{-1} 3366, 3241, 3180, 2981, 2956, 2883, 1679 (C=O), 1616, 1386, 1333, 1109, 829 and 747; δ_H(400 MHz) 0.89 and 0.91 (each 3H, each s, 8-H_3 and 9-H_3), 1.14 (3H, d, *J* 5.5, 2′-CH_3), 1.20 (3H, d, *J* 6.0, 4′-H_3), 1.23-1.44 (2H, m, 5-*H*H and 6-*H*H), 1.78-1.92 (5H, m, 3-H_2, 4-H, 5-H*H* and 6-H*H*), 3.29 (1H, app. quint., *J* 7.0, 2′-H), 3.39 (1H, d, *J* 14.0, 10-*H*H), 3.46 (1H, d, *J* 14.0, 10-H*H*), 3.81-3.85 (1H, m, 3′-H), 4.38 (1H, d, *J* 11.5, C*H*HPh), 4.56 (1H, d, *J* 11.5, CH*H*Ph), 7.28-7.32 (5H, m, 5 × Ar-H); δ_C(100 MHz) 12.7 and 16.5 (2′-CH_3 and C4′), 19.88 and 20.6 (C-8 and C-9), 26.5 and 32.8 (C-5 and C-6), 38.4 (C-3), 44.7 (C-4), 46.0 (C-2′), 47.6 and 48.2 (C-1 and C-7), 53.2 (C-10), 65.2 (C-2), 71.1 (*C*H_2Ph), 78.1 (C-3′), 127.3 (Ar-C), 128.1 (2 × Ar-C), 128.1 (2 × Ar-C), 138.6 (Ar-C_{ipso}), 174.8 (C-1′); *m/z* (CI) 406 (MH^+, 50%), 306 (100), 298 (30), 216 (34), 135 (33), 119 (26) and 91 (98); Found (CI): 406.2047 (MH^+), ($C_{22}H_{32}NO_4S$ requires 406.2052).

(3*R*,4*S*)-4-Benzyloxy-3-methylpent-1-ene 135

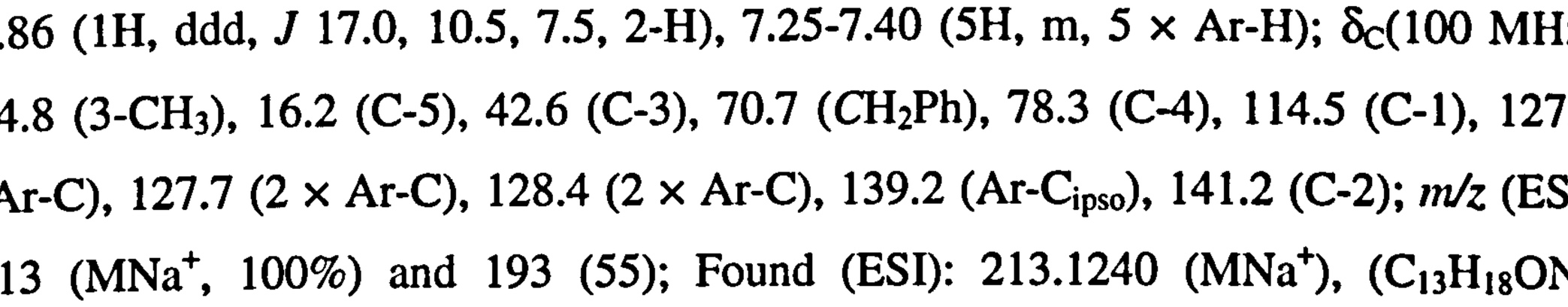


134 135

Diisobutylaluminium hydride (1.0 M in toluene, 3.71 mL, 3.71 mmol) was added dropwise in 3 portions at 15 minute intervals to benzyl ether **134** (1.00 g, 2.46 mmol) in dry DCM (10 mL) under an atmosphere of nitrogen at -78 °C. The reaction was stirred for 1 hour and then a further addition of diisobutylaluminum hydride (1.0 M in toluene, 0.5 mL, 0.5 mmol) was made. The reaction was stirred for 30 minutes and was then quenched with saturated aqueous potassium sodium L-tartrate tetrahydrate solution (50 mL). The solution was stirred vigorously overnight and was then extracted with DCM (3 × 100 mL), washed with brine (20 mL), dried over magnesium sulfate and concentrated *in vacuo*. The resulting oil was triturated with

petroleum ether 40-60 °C for several minutes and filtered to remove the precipitated auxiliary. The filtrate was concentrated *in vacuo* to yield a pale yellow oil. Meanwhile, a solution of methyltriphenylphosphonium bromide (4.13 g, 11.43 mmol) and sodium hydride (60% dispersion in oil prewashed with dry hexane, 0.98 g, 24.6 mmol) in dry THF (25 mL) under an atmosphere of nitrogen was gently heated with stirring until a green/yellow tinge was observed. The solution was stirred overnight at room temperature and the solids were then allowed to settle. The bright yellow solution was then transferred *via* cannula into a solution of the aldehyde in dry THF (3 mL) and left to stir at room temperature under an atmosphere of nitrogen for 3 hours. The solvent was then removed *in vacuo* and the resulting yellow residue was purified by flash column chromatography (SiO_2, 0-1% ethyl acetate/petroleum ether 40-60 °C, dry loaded) to give *alkene* **135** (0.22 g, 46%) as a colourless oil. $[\alpha]_D^{23}$ +16.8 (*c* 3.04, $CHCl_3$); ν_{max}(neat)/cm^{-1} 2973, 2869, 1454, 1373, 1094, 1067, 911, 733, 695; δ_H(400 MHz) 1.07 (3H, d, *J* 7.0, 3-CH_3), 1.15 (3H, d, *J* 6.5, 5-H_3), 2.45 (1H, m, 3-H), 3.48 (1H, qd, *J* 6.5, 5.0, 4-H), 4.51 and 4.60 (each 1H, each d, *J* 12.0, CH_2Ph), 5.08 (1H, ddd, *J* 10.5, 2.0, 1.0, 1-*H*H), 5.09 (1H, ddd, *J* 17.0, 2.0, 1.0, 1-H*H*), 5.86 (1H, ddd, *J* 17.0, 10.5, 7.5, 2-H), 7.25-7.40 (5H, m, 5 × Ar-H); δ_C(100 MHz) 14.8 (3-CH_3), 16.2 (C-5), 42.6 (C-3), 70.7 (CH_2Ph), 78.3 (C-4), 114.5 (C-1), 127.5 (Ar-C), 127.7 (2 × Ar-C), 128.4 (2 × Ar-C), 139.2 (Ar-C_{ipso}), 141.2 (C-2); *m/z* (ESI) 213 (MNa^+, 100%) and 193 (55); Found (ESI): 213.1240 (MNa^+), ($C_{13}H_{18}ONa$ requires 213.1250).

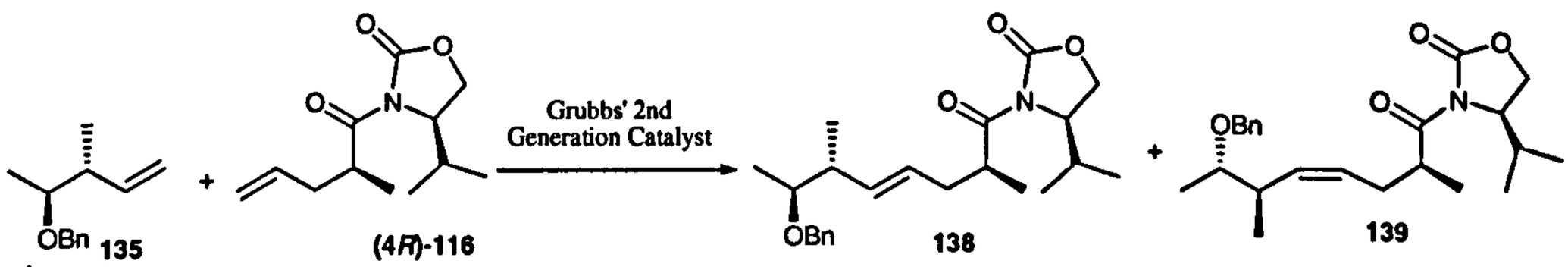
(4*S*)-*N*-((2*R*,4*E*,6*R*,7*S*)-7-Benzyloxyoct-2,6-dimethyl-4-enoyl)-4-isopropyl-2-oxazolidinone 136



To a solution of Grubbs' second generation catalyst (66.2 mg, 0.076 mmol) in dry toluene (5.5 mL) was added a premixed solution of alkene **135** (0.30 g, 1.53 mmol) and alkene **(4*S*)-116** (0.17 g, 0.76 mmol) in dry toluene (7 mL). The reaction was stirred overnight at 50 °C and then solvents were removed *in vacuo* to give a purple-black oil. Purification by flash column chromatography (SiO_2, 2.5-5% ethyl acetate/petroleum ether 40-60 °C) afforded an inseparable 6:1 mixture of *alkenes* **136**

and **137** (0.12 g, 41%) as a colourless oil. $[\alpha]_D^{22}$ (of 6:1 mixture) +18.2 (*c* 2.96, $CHCl_3$); ν_{max}(neat)/cm^{-1} 2969, 2917, 1777 (C=O), 1700 (C=O), 1495, 1454, 1386, 1373, 1235, 1203, 1094, 1056, 991, 734, 698; δ_H(400 MHz) (major isomer) 0.86 and 0.90 (each 3H, each d, *J* 7.0, $CH(CH_3)_2$), 0.99 (3H, d, *J* 7.0, 6′-CH_3), 1.10 (3H, d, *J* 6.0, 8′-H_3), 1.12 (3H, d, *J* 7.0, 2′-CH_3), 2.13 (1H, app. dt, *J* 14.0, 7.0, 3′-*H*H), 2.26-2.42 (2H, m, $CH(CH_3)_2$ and 6′-H), 2.48 (1H, app. dt, *J* 14.0, 7.0, 3′-H*H*), 3.42 (1H, qd, *J* 6.0, 4.5, 7′-H), 3.80 (1H, app. sext., *J* 7.0, 2′-H), 4.19 (1H, dd, *J* 9.0, 3.0, 5-*H*H), 4.26 (1H, dd, *J* 9.0, 8.5, 5-H*H*), 4.45 (1H, m, 4-H), 4.46 (1H, d, *J* 12.0, C*H*HPh), 4.55 (1H, d, *J* 12.0, CH*H*Ph), 5.39 (1H, dt, *J* 15.5, 7.0, 4′-H), 5.48 (1H, dd, *J* 15.5, 7.0, 5′-H), 7.30-7.36 (5H, m, 5 × Ar-H); δ_C(100 MHz) (major isomer) 14.7 ($CHCH_3$), 15.2 (6′-CH_3), 16.0 and 16.1 (2′-CH_3 and C-8′), 17.9 ($CHCH_3$), 28.4 ($CH(CH_3)_2$), 37.0 (C-3′), 37.7 (C-2′), 41.4 (C-6′), 58.4 (C-4), 63.1 (C-5), 70.6 (CH_2Ph), 78.4 (C-7′), 126.7 (C-4′), 127.3 (Ar-C), 127.5 (2 × Ar-C), 128.2 (2 × Ar-C), 135.5 (C-5′), 139.1 (Ar-C_{ipso}), 153.7 (C-2), 176.5 (C-1′); *m/z* (ESI) 410 (MNa^+, 100%) and 137 (11); Found (ESI): 410.2308 (MNa^+), ($C_{23}H_{33}O_4NNa$ requires 410.2302).

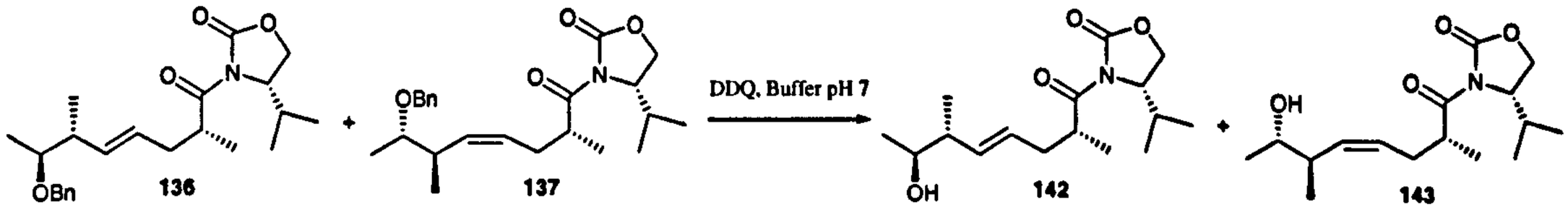
(4*R*)-*N*-((2*S*,4*E*,6*R*,7*S*)-2,6-Dimethyl-7-benzyloxyoct-4-enoyl)-4-isopropyl-2-oxazolidinone 138



To a solution of Grubbs' second generation catalyst (12.2 mg, 0.014 mmol) in dry toluene (2 mL) was added a premixed solution of alkene **135** (0.11 g, 0.56 mmol) and alkene **(4*R*)-116** (0.06 g, 0.28 mmol) in dry toluene (2.5 mL). The reaction was stirred overnight at 50 °C and then solvents were removed *in vacuo* to give a purple-black oil. Purification by flash column chromatography (SiO_2, 2.5-5% ethyl acetate/petroleum ether 40-60 °C) afforded a 6:1 mixture of *alkenes* **138** and **139** (0.04 g, 40%) as a colourless oil. $[\alpha]_D^{24}$ (of 6:1 mixture) -23.2 (*c* 1.12, $CHCl_3$); ν_{max}(neat)/cm^{-1} 2967, 2920, 2860, 1778 (C=O), 1701 (C=O), 1454, 1385, 1300, 1236, 1203, 1094, 966, 737, 698; δ_H(400 MHz) (major isomer) 0.86 and 0.91 (each 3H, each d, *J* 7.0, $CH(CH_3)_2$), 1.00 (3H, d, *J* 7.0, 6′-CH_3), 1.10 (3H, d, *J* 6.5, 8′-H_3), 1.13 (3H, d, *J* 7.0, 2′-CH_3), 2.14 (1H, app. dt, *J* 13.5, 7.0, 3′-*H*H), 2.26-2.44 (2H, m, $CH(CH_3)_2$

and 6′-H), 2.49 (1H, app. dt, *J* 13.5, 7.0, 3′-H*H*), 3.42 (1H, qd, *J* 6.5, 4.5, 7′-H), 3.80 (1H, app. sext., *J* 7.0, 2′-H), 4.20 (1H, dd, *J* 9.0, 3.0, 5-*H*H), 4.27 (1H, dd, *J* 9.0, 8.5, 5-H*H*), 4.47 (1H, m, 4-H), 4.48 (1H, d, *J* 12.0, C*H*HPh), 4.56 (1H, d, *J* 12.0, CH*H*Ph), 5.39 (1H, dt, *J* 15.5, 7.0, 4′-H), 5.49 (1H, dd, *J* 15.5, 7.0, 5′-H), 7.30-7.36 (5H, m, 5 × Ar-H); δ_C(100 MHz) (major isomer) 14.7 (CH*C*H_3), 14.9 (6′-CH_3), 15.9 and 16.0 (2′-CH_3 and C-8′), 18.0 (CH*C*H_3), 28.4 (*C*HCH_3), 37.0 (C-3′), 37.7 (C-2′), 41.3 (C-6′), 58.4 (C-4), 63.1 (C-5), 70.6 (*C*H_2Ph), 78.3 (C-7′), 126.6 (C-4′), 127.3 (Ar-C), 127.5 (2 × Ar-C), 128.2 (2 × Ar-C), 135.5 (C-5′), 139.1 (Ar-C_{ipso}), 153.7 (C-2), 176.5 (C-1′); *m/z* (ESI) 410 (MNa^+, 100%); Found (ESI): 410.2312 (MNa^+), ($C_{23}H_{33}O_4NNa$ requires 410.2302).

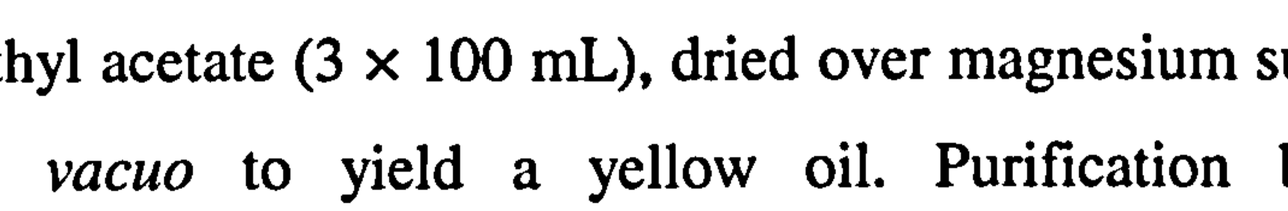
(4*S*)-*N*-((2*R*,4*E*,6*R*,7*S*)-2,6-Dimethyl-7-hydroxyoct-4-enoyl)-4-isopropyl-2-oxazolidinone 142



To benzyl ethers **136** and **137** (0.11 g, 0.28 mmol) in DCM (5.5 mL) and buffer pH 7 solution (1.0 M solution prepared by dissolving sodium dihydrogen phosphate (0.5999 g, 5 mmol) and disodium hydrogen phosphate (0.7098 g, 5 mmol) in water (10 mL) and adjusting the pH to 7 by addition of the relevant substrate, 1.0 mL) was added 2,3-dichloro-5,6-dicyanobenzoquinone (0.048 g, 0.21 mmol). The mixture was shaken at 250 rpm for 3 days. Ethyl acetate (30 mL) and sodium hydrogen carbonate solution (10 mL) were added. The layers were separated and the aqueous phase was extracted with ethyl acetate (2 × 30 mL). The combined organic phases were washed with brine (10 mL), dried over sodium sulfate, filtered and concentrated *in vacuo* to give a dark red residue. Purification by flash column chromatography (SiO_2, 20% ethyl acetate/petroleum 40-60 °C) afforded an inseparable 6:1 mixture of *alcohols* **142** and **143** (0.06 g, 71%) as a colourless oil. $[\alpha]_D^{25}$(of 6:1 mixture) +61.2 (*c* 1.77, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3499 (OH), 2966, 2933, 2877, 1774 (C=O), 1698 (C=O), 1457, 1385, 1300, 1234, 1200, 1092, 1054, 966, 775, 691; δ_H(400 MHz) (major isomer) 0.87 and 0.91 (each 3H, each d, *J* 7.0, CH(C*H*$_3$)$_2$), 0.95 (3H, d, *J* 7.0, 6′-CH_3), 1.13 (3H, d, *J* 7.0, 2′-CH_3), 1.15 (3H, d, *J* 6.0, 8′-H_3), 1.97 (1H, br s, OH), 2.05 (1H, m, 6′-H), 2.20 (1H, dddd, *J* 14.0, 7.0, 6.5, 1.0, 3′-*H*H), 2.31 (1H, sept.d, *J* 7.0, 4.0, C*H*(CH$_3$)$_2$), 2.47

(1H, dtd, *J* 14.0, 6.5, 1.0, 3′-H*H*), 3.44 (1H, app. quint., *J* 6.5, 7′-H), 3.86 (1H, app. sext., *J* 7.0, 2′-H), 4.20 (1H, dd, *J* 9.0, 3.0, 5-*H*H), 4.26 (1H, dd, *J* 9.0, 8.5, 5-H*H*), 4.44 (1H, ddd, *J* 8.5, 4.0, 3.0, 4-H), 5.31 (1H, ddt, *J* 15.5, 8.5, 1.0, 5′-H), 5.51 (1H, dtd, *J* 15.5, 6.5, 0.5, 4′-H); δ_C(100 MHz) (major isomer) 14.6 (CH*C*H$_3$), 15.8 and 16.4 (6′-CH$_3$ and C-8′), 18.0 (CH*C*H$_3$), 20.0 (2′-CH$_3$), 28.4 (*C*H(CH$_3$)$_2$), 36.7 (C-3), 37.4 (C-2′), 45.1 (C-6′), 58.4 (C-4), 63.1 (C-5), 70.8 (C-7′), 128.6 (C-4′), 135.2 (C-5′), 153.7 (C-2), 176.3 (C-1′); *m/z* (ESI) 320 (MNa$^+$, 100%), 280 (9) and 107 (11); Found (ESI): 320.1829 (MNa$^+$), (C$_{16}$H$_{27}$NO$_4$Na requires 320.1832).

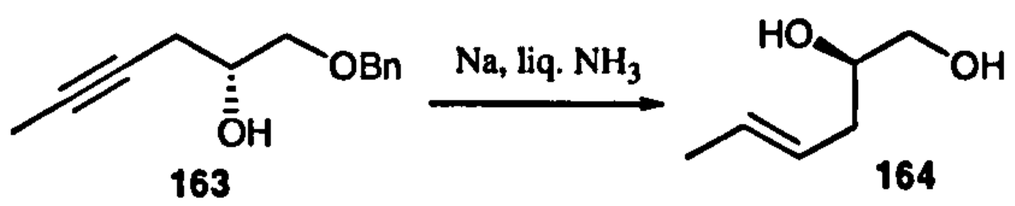
(*R*)-1-Benzyloxyhex-4-yn-2-ol 163[99]



162 **163**

Propyne (≈20 mL) was condensed into a 2-necked round bottom flask connected to a cold finger at -78 °C. *n*-Butyllithium (20 mL, 50 mmol) was then added dropwise followed by boron trifluoride diethyl etherate (5.1 mL, 36.00 mmol), epoxide **162** (5.0 mL, 32.80 mmol) and dry THF (20 mL) and the reaction was left at -78 °C for 3 hours. The reaction was then quenched using saturated aqueous ammonium chloride solution (50 mL) and was allowed to warm to room temperature. The solution was extracted with ethyl acetate (3 × 100 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield a yellow oil. Purification by flash column chromatography (SiO_2, 10-20% ethyl acetate/petroleum ether 40-60 °C) furnished alkyne **163** (4.51 g, 67%) as a colourless oil. Although a known compound, (*R*)-1-benzyloxy-hex-4-yn-2-ol has not been previously characterised. $[\alpha]_D^{22}$ +8.16 (*c* 0.98, CH_2Cl_2); υ_{max}(neat)/cm^{-1} 3471 (OH), 3364 (C≡C), 2920, 2869, 1074, 734 and 698; δ_H(400 MHz) 1.77 (3H, t, *J* 2.5, 6-H$_3$), 2.39 (2H, dq, *J* 5.5, 3.0, 3-H$_2$), 2.50 (1H, d, *J* 4.5, OH), 3.48 (1H, dd, *J* 9.5, 7.0, 1-*H*H), 3.59 (1H, dd, *J* 9.5, 4.0, 1-H*H*), 3.92 (1H, m, 2-H), 4.56 (2H, s, PhC*H*$_2$O), 7.27-7.37 (6H, m, 5 × Ar-H); δ_C(100 MHz) 3.5 (C-6), 23.9 (C-3), 69.1 (C-2), 73.1 and 73.4 (C-1 and Ph*C*H$_2$O), 74.7 (C-5), 78.1 (C-4), 127.7 (2 × Ar-C), 127.8 (Ar-C), 128.4 (2 × Ar-C), 137.9 (Ar-C$_{ipso}$); *m/z* (CI) 204 (M$^+$, 20%), 187 (30), 169 (27), 157 (20), 145 (30), 143 (55), 127 (68) and 91 (100); Found (CI): 203.0990 (M$^+$ - H$^+$), (C$_{13}$H$_{15}$O$_2$ requires 203.1078).

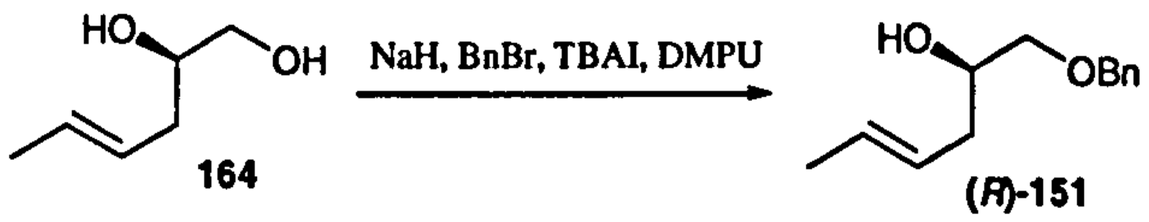
(2*R*,4*E*)-Hex-4-en-1,2-diol 164[99]



163 164

Homopropargyl alcohol **163** (1.00 g, 4.90 mmol) was dissolved in dry THF (1.5 mL) and cooled to -44 °C. Liquid ammonia (≈40 mL) was condensed into the flask using a cold finger at -78 °C. Sodium metal (prewashed in hexane, ≈1.00 g, 43.4 mmol) was added in pieces until the colour of the solution remained indigo for >50 minutes. The reaction was then quenched with solid ammonium chloride and the mixture was allowed to warm to room temperature. When all the ammonia had evaporated off, water (40 mL) was added to the mixture and the solution was extracted with diethyl ether (3 × 50 mL). The combined organic extracts were washed with water (20 mL) and brine (20 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The resulting yellow oil was purified by flash column chromatography (SiO_2, 10-100% ethyl acetate/petroleum ether 40-60 °C) to give diol **164** (0.34 g, 61%) as a pale yellow oil. Although a known compound, diol **164** has not previously been characterised. $[\alpha]_D^{24}$ -5.5 (*c* 1.45, $CHCl_3$); υ_{max}(neat)/cm^{-1} 3337 (OH), 2919, 1668 (C=C), 1062, 1028, 966 (C=C); δ_H(400 MHz) 1.69 (3H, qd, *J* 6.5, 1.0, 6-H_3), 2.10-2.32 (4H, m, 3-H_2 and 2 × OH), 3.48 (1H, m, 2-H), 3.63-3.76 (2H, m, 1-H_2), 5.44 (1H, dtq, *J* 15.0, 7.0, 1.5, 4-H), 5.59 (1H, dqt, *J* 15.0, 6.5, 1.0, 5-H); δ_C(100 MHz) 18.1 (CH_3), 36.7 (C-3), 66.3 (C-1), 71.5 (C-2), 126.3 and 129.2 (C-4 and C-5); *m/z* (EI) 117 (MH^+, 6%), 98 (32), 91 (36), 86 (82), 84 (100), 67 (34), 61 (50) and 56 (67); Found (CI): 99.0805 (M^+ - H_2O), ($C_6H_{11}O$ requires 99.0810).

(2*R*,4*E*)-1-Benzyloxy-hex-4-en-2-ol (*R*)-151[186]

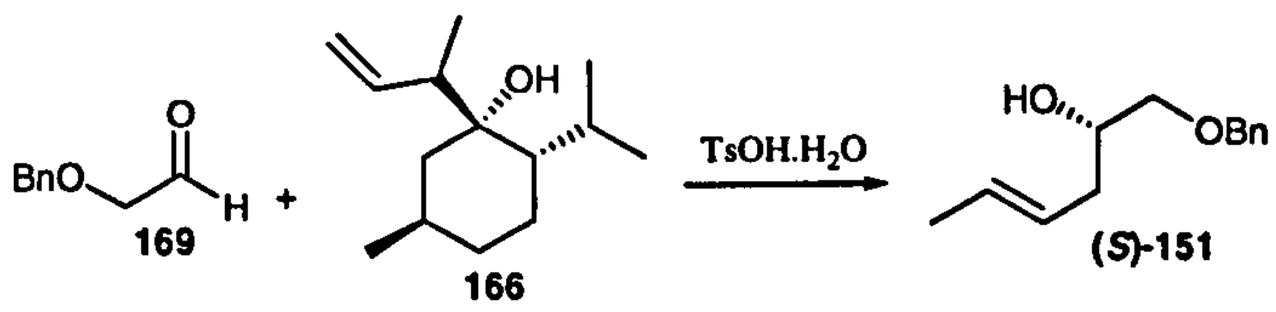


164 (*R*)-151

Sodium hydride (60% dispersion oil prewashed in hexane, 0.12 g, 4.88 mmol) was suspended in dry THF (15 mL) and cooled to 0 °C under an atmosphere of nitrogen. Diol **164** (0.34 g, 2.93 mmol) in dry THF (5 mL) was added dropwise and the solution was stirred for 30 minutes. 1,3-Dimethyl-3,4,5,6-tetrahydro-2-(1H)-pyrimidinone (0.63 g, 0.49 mmol), tetrabutylammonium iodide (0.18 g, 0.49 mmol) and benzyl bromide (0.84 g, 4.88 mmol) were added and the solution was stirred at room temperature for 23 hours. The reaction was then quenched with saturated aqueous

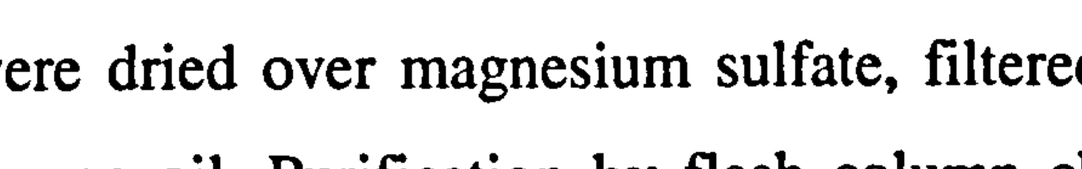
ammonium chloride solution (50 mL). The layers were separated and the aqueous layer was extracted with DCM (3 × 100 mL). The combined organic extracts were then dried over magnesium sulfate, filtered and concentrated *in vacuo*. Purification by flash column chromatography (SiO_2, 10% ethyl acetate/petroleum ether 40-60 °C) yielded homoallylic alcohol (***R*)-151** (0.06 g, 10%) as a colourless oil. $[\alpha]_D^{22}$ +10.8 (*c* 1.48, Et_2O), $[\alpha]_D$ lit.[186] +1.52 (*c* 4.70, Et_2O); δ_H(400 MHz) 1.67 (3H, qd, *J* 6.0, 1.0, 6-H_3), 2.19 (2H, app. tt, *J* 7.0, 1.0, 3-H_2), 2.33 (1H, d, *J* 3.5, OH), 3.36 (1H, dd, *J* 9.5, 3.5, 1-*H*H), 3.50 (1H, dd, *J* 9.5, 7.5, 1-H*H*), 3.83 (1H, m, 2-H), 4.55 (2H, s, PhCH_2O), 5.42 (1H, dtq, *J* 15.0, 7.0, 1.0, 4-H), 5.53 (1H, dqt, *J* 15.0, 6.5, 1.0, 5-H), 7.28-7.38 (5H, m, 5 × Ar-H); δ_C(100 MHz) 18.0 (CH_3), 36.7 (C-3), 70.1 (C-2), 73.4 and 74.0 (C-1 and C-2), 126.5 (C-4 or C-5), 127.8 (2 coincident peaks, 2 × Ar-C and C-4 or C-5), 128.4 (3 × Ar-C), 138.1 (Ar-C_{ipso}); *m/z* (EI) 206 (M^+, 22%), 151 (41), 107 (52), 91 (100), 86 (60), 84 (77), 65 (56) and 57 (67).

(2*S*,4*E*)-1-Benzyloxyhex-4-en-2-ol (*S*)-151[186]



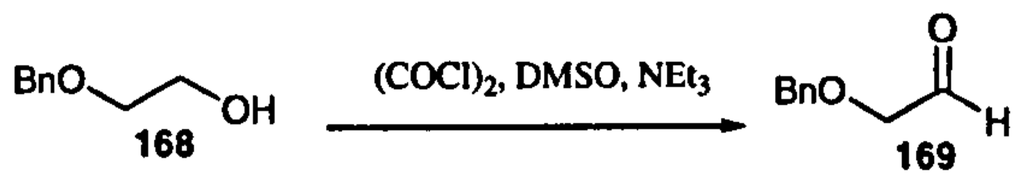
p-Toluenesulfonic acid monohydrate (0.31 g, 1.62 mmol) was added to a solution of aldehyde **169** (2.50 g, 16.64 mmol) and alcohol **166** (7.00 g, 33.27 mmol) in dry DCM (80 mL) at room temperature. The reaction was stirred for 20 hours and was then quenched with triethylamine (1.00 mL) and saturated sodium hydrogen carbonate solution (50 mL). After 15 minutes of vigorous stirring the layers were separated and the aqueous layer was extracted with DCM (3 × 100 mL). The combined extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield an orange oil. Purification by flash column chromatography (SiO_2, 10% ethyl acetate/petroleum ether 40-60 °C) yielded alcohol (***S*)-151** (2.02 g, 59%) as a pale yellow oil. $[\alpha]_D^{22}$ -2.00 (*c* 4.00, Et_2O), $[\alpha]_D$ lit.[186] +1.52 (*c* 4.70, Et_2O) for enantiomer; Spectral data as above.

2-Benzyloxyethanol 168[187]



Sodium hydride (60% dispersion in oil prewashed in hexane, 2.40 g, 60.00 mmol) was suspended in dry THF (70 mL) and cooled to 0 °C under an atmosphere of nitrogen. After 10 minutes, ethane-1,2-diol **167** (34.36 g, 553.50 mmol), DMPU (0.75 mL, 6.20 mmol), TBAI (2.00 g, 5.40 mmol) and benzyl bromide (7.0 mL, 59.00 mmol) were added slowly and the mixture was stirred at room temperature for 21 hours. The reaction was quenched with saturated aqueous ammonium chloride solution (50 mL). The layers were separated and the aqueous layer was extracted with DCM (3 × 50 mL). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield an orange oil. Purification by flash column chromatography (SiO_2, 50% ethyl acetate/petroleum ether 40-60 °C) furnished alcohol **168** (7.48 g, 83%) as a yellow oil. δ_H(400 MHz) 2.38 (1H, br s, OH), 3.57 (2H, t, *J* 4.5, 2-H_2), 3.73 (2H, m, 1-H_2), 4.55 (2H, s, CH_2Ph), 7.26-7.38 (5H, m, 5 × Ar-H); δ_C(100 MHz) 61.8 (C-1), 71.4 (C-2), 73.2 (CH_2Ph), 127.8 (2 × Ar-C), 128.4 (3 × Ar-C), 137.9 (Ar-C_{ipso}).

2-Benzyloxyethanal 169[188]

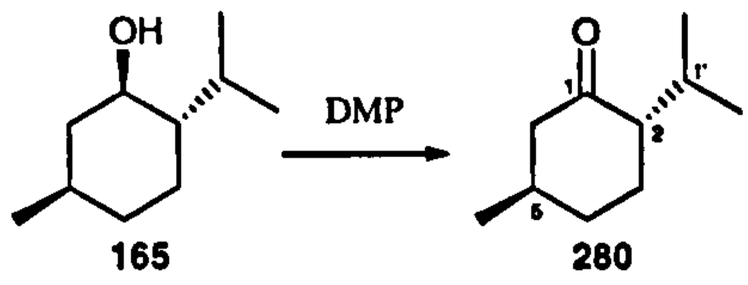


Dimethylsulfoxide (12.2 mL, 171.26 mmol) was added slowly to a solution of oxalyl chloride (7.46 mL, 85.66 mmol) in dry DCM (150 mL) at -78 °C under an atmosphere of nitrogen. The mixture was stirred for 20 minutes and then a solution of 2-benzyloxyethanol **168** (10.00 g, 65.87 mmol) in dry DCM (30 mL) was added dropwise *via* cannula. The reaction mixture was stirred for 1 hour and then triethylamine (freshly distilled over calcium hydride, 10.4 mL, 74.70 mmol) was added and the reaction was allowed to warm to room temperature. After a further 20 minutes, saturated aqueous ammonium chloride solution (200 mL) was added and the layers were separated. The aqueous phase was extracted with DCM (3 × 100 mL) and the combined organic extracts were washed with hydrochloric acid (1.0 M, 50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* at 0 °C to yield a yellow oil. Purification by flash column chromatography (SiO_2, 10% ethyl acetate/petroleum ether 40-60 °C) gave aldehyde **169** (4.31 g, 44%) as a colourless oil. δ_H(400 MHz) 4.10 (2H, s, 2-H_2), 4.62 (2H, CH_2Ph), 7.27-7.43 (5H, m, 5 × Ar-H),

9.72 (1H, s, CHO); δ_C(100 MHz) 73.7 ($COCH_2$), 75.3 (C-2), 128.0 (Ar-C), 128.2 (2 × Ar-C), 128.6 (2 × Ar-C), 136.8 (Ar-C_{ipso}), 200.5 (C-1).

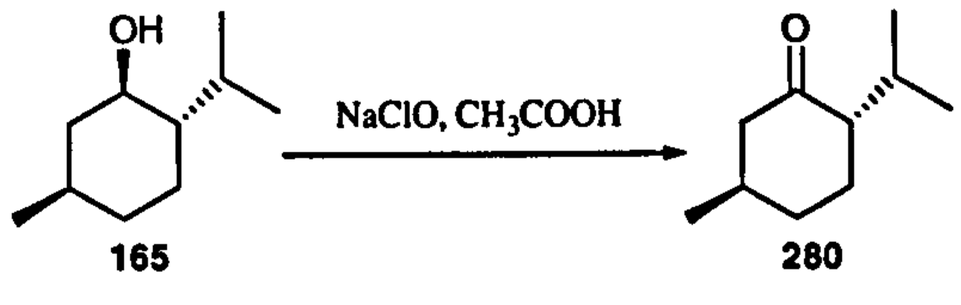
(2*S*,5*R*)-Menthone 280[189]

(a) *via* Dess-Martin Oxidation



A solution of (1*R*,2*S*,5*R*)-menthol **165** (1.08 g, 6.93 mmol) in dry DCM (20 mL) was added dropwise to Dess-Martin periodinane (15% w/w in DCM, 20.0 mL, 9.60 mmol,) in dry DCM (15 mL) at room temperature under an atmosphere of nitrogen. The reaction mixture was stirred for 4 hours and was then added to sodium hydroxide solution (1.0 M, 50 mL). The aqueous layer was extracted with diethyl ether (3 × 50 mL) and then the combined extracts were washed with water (50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield (2*S*,5*R*)-menthone **280** (1.02 g, 97%) as a colourless oil. This was used without further purification. $[\alpha]_D^{23}$ -25.4 (neat), lit.[189] $[\alpha]_D$ -25.3 (neat); δ_H(400 MHz) 0.86 and 0.92 (each 3H, each d, *J* 7.0, 1'-$(CH_3)_2$), 1.01 (3H, d, *J* 6.5, 5-CH_3), 1.15-1.45 (2H, m, 3-*H*H and 4-*H*H), 1.75-2.20 (6H, m, 1'-H, 2-H, 3-H*H*, 4-H*H*, 5-H, 6-*H*H), 2.36 (1H, ddd, *J* 12.5, 3.5, 2.5, 6-H*H*); δ_C(100 MHz) 18.7 and 21.2 (2 × 1'-CH_3), 22.3 (5-CH_3), 25.9 (C-1' or C-5), 27.9 and 33.9 (C-3 and C-4), 35.9 (C-1' or C-5), 50.9 (C-6), 55.9 (C-2), 212.4 (C-1).

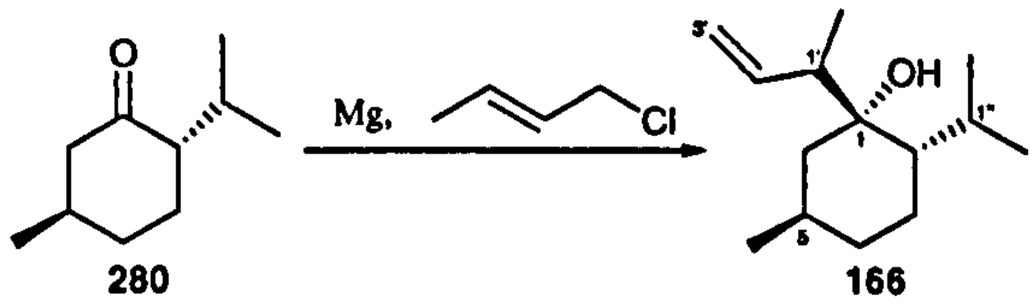
(b) *via* sodium hypochlorite oxidation



Aqueous sodium hypochlorite (138 mL, 179.6 mmol, ≈ 1.3 M) was added slowly to a solution of (1*R*, 2*S*, 5*R*)-menthol **165** (20.00 g, 127.80 mmol) in acetic acid (96 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2 hours and was then quenched by addition of sodium hydrogen sulfite solution until the yellow solution had become white in colour. Brine (60 mL) and diethyl ether (60 mL) were added and the layers were separated. The aqueous layer was extracted with diethyl ether (3 × 200 mL) and the combined organic layers were then washed with saturated aqueous

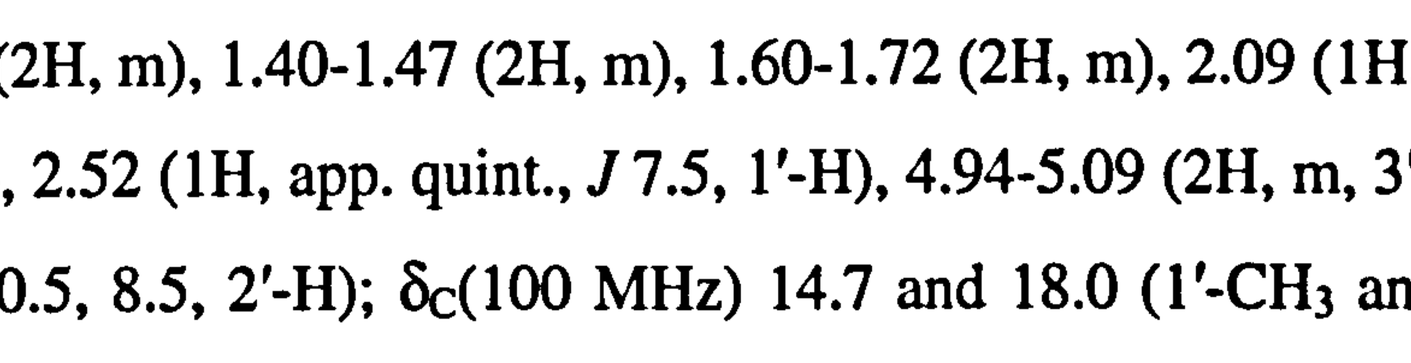
sodium hydrogen carbonate solution (50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to gave (2*S*,5*R*)-menthone **280** (38.84 g, 98%) as a colourless oil. This was used without further purification. Spectral data as above.

(1*S*,2*S*,5*R*,1′*R*)-1-(1-Methylallyl)menthol 166[102]



A solution of crotyl chloride (5.94 mL, 56.80 mmol) in dry THF (40 mL) was added slowly to freshly ground magnesium turnings (1.38 g, 56.80 mmol) at room temperature under an atmosphere of nitrogen. When addition was complete the reaction was maintained at reflux for 25 minutes. The solution was then cooled to 0 °C and (2*S*,5*R*)-menthone **280** (5.16 g, 33.4 mmol) in dry THF (12 mL) was added dropwise and the mixture was stirred for 2 hours at 0 °C. The reaction was quenched with saturated aqueous ammonium chloride solution (200 mL). Water (50 mL) was added and the biphasic solution was filtered prior to extraction with ethyl acetate (3 × 100 mL). The combined extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield pale yellow oil. Purification by flash column chromatography (SiO_2, 1% ethyl acetate/petroleum ether 40-60 °C) gave alcohol **166** (3.57 g, 51%) as a colourless oil. $[\alpha]_D^{24}$ +26.9 (*c* 1.02, $CHCl_3$), lit.[102] $[\alpha]_D$ +27.4 (*c* 1.00, $CHCl_3$); δ_H(400 MHz) 0.79 (3H, d, *J* 6.5, 5-CH_3), 0.68-1.09 (2H, m), 0.84 (3H, d, *J* 2.5, 1″-CH_3), 0.85 (3H, d, *J* 2.5, 1″-CH_3), 0.89 (3H, d, *J* 7.5, 1′-CH_3), 1.18 (1H, m), 1.19-1.31 (2H, m), 1.40-1.47 (2H, m), 1.60-1.72 (2H, m), 2.09 (1H, app. quint.d, *J* 7.0, 1.5, 1″-H), 2.52 (1H, app. quint., *J* 7.5, 1′-H), 4.94-5.09 (2H, m, 3′-H_2), 5.81 (1H, ddd, *J* 18.0, 10.5, 8.5, 2′-H); δ_C(100 MHz) 14.7 and 18.0 (1′-CH_3 and 5-CH_3), 20.6 (C-3), 22.6 and 23.4 (2 × 1″-CH_3), 25.0 (C-1″), 27.6 (C-5), 35.3 (C-4), 41.5 (C-6), 45.2 (C-1′), 46.0 (C-2), 76.2 (C-1), 116.6 (C-3′), 140.8 (C-2′).

Ethyl (2*E*,4*R*,5*S*)-5-Benzyloxy-4-methylhex-2-enoate 171

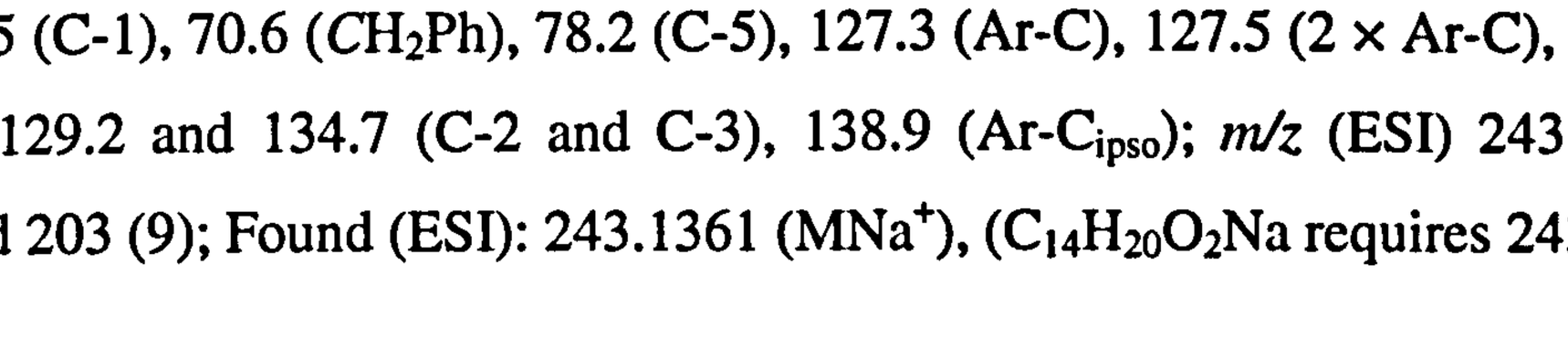


Benzyl ether **134** (2.00 g, 4.92 mmol) was dissolved in dry DCM (40 mL) under an atmosphere of nitrogen and cooled to -78 °C. Diisobutylaluminium hydride (1.0 M in hexanes, 4.9 mL, 4.92 mmol) was added dropwise in 3 portions at 15 minute intervals. After 1.5 hours a further addition of diisobutylaluminium hydride (2.5 mL, 2.46 mmol) was required. After 2.5 hours the reaction was quenched with saturated aqueous potassium sodium L-tartrate tetrahydrate solution (20 mL). The reaction was warmed to room temperature and stirred vigorously overnight to break up the aluminium complex. The mixture was then extracted with DCM (3 × 50 mL) and the combined organic layers were washed with water (5 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* at 0 °C. The resulting oil was triturated with petroleum ether 40-60 °C and filtered to remove the precipitated auxiliary. The filtrate was concentrated *in vacuo* to yield a colourless oil. This was then dissolved in dry THF (6 mL) and added dropwise to a solution of triethyl phosphonoacetate (2.2 mL, 10.82 mmol) and *n*-butyllithium (2.5 M in hexanes, 4.2 mL, 10.82 mmol) in dry THF (60 mL) which had been stirred for 10 minutes at 0 °C under an atmosphere of nitrogen. The resulting solution was stirred at room temperature for 20 hours and was then quenched with water (50 mL). The solution was extracted with ethyl acetate (3 × 150 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. Purification by flash column chromatography (SiO_2, 5% ethyl acetate/petroleum ether 40-60 °C) gave *ester* **171** (1.70 g, 66%) as a colourless oil. $[\alpha]_D^{20}$ +22.7 (*c* 2.29, $CHCl_3$); ν_{max}(neat)/cm^{-1} 2976, 2932, 2359, 2333, 1715 (C=O), 1652 (C=C), 1454, 1368, 1183, 1094, 1038, 984, 734, 697; δ_H(400 MHz) 1.08 (3H, d, *J* 7.0, 4-CH_3), 1.15 (3H, d, *J* 6.5, 6-H_3), 1.29 (3H, t, *J* 7.0, OCH_2CH_3), 2.55 (1H, m, 4-H), 3.50 (1H, dq, *J* 6.5, 5.5, 5-H), 4.19 (2H, q, *J* 7.0, OCH_2CH_3), 4.46 (1H, d, *J* 11.5, C*H*HPh), 4.58 (1H, d, *J* 11.5, CH*H*Ph), 5.83 (1H, dd, *J* 16.0, 1.0, 2-H), 6.98 (1H, dd, *J* 16.0, 7.5, 3-H), 7.20-7.38 (5H, m, 5 × Ar-H); δ_C(100 MHz) 14.3 (OCH_2CH_3), 14.6 (4-CH_3), 16.5 (C-6), 41.8 (C-4), 60.2 (OCH_2CH_3), 70.7 (CH_2Ph), 77.5 (C-5), 121.3 (C-2), 127.5 (Ar-C), 127.6 (2 × Ar-C), 128.3 (2 × Ar-C), 138.6 (Ar-C_{ipso}), 151.1 (C-3), 166.7 (C-1); *m/z* (CI) 263 (MH^+, 19%), 235 (24), 221 (20), 181 (96), 155 (38), 127 (21), 91 (100) and 84 (25); Found (CI): 263.1639 (MH^+), ($C_{16}H_{23}O_3$ requires 263.1647).

(2*E*,4*R*,5*S*)-5-Benzyloxy-4-methylhex-2-enol 172

Ethyl ester **171** (1.07 g, 4.08 mmol) was dissolved in dry DCM (55 mL) under an atmosphere of nitrogen at -78 °C. Diisobutylaluminium hydride (1.0 M in hexanes, 9.0 mL, 9.0 mmol) was added dropwise and the solution was stirred at -78 °C for 2 hours. The reaction was then quenched with saturated aqueous potassium sodium L-tartrate tetrahydrate solution (40 mL) and was stirred vigorously at room temperature for 3 hours to break up the aluminium complex. The organic layer was removed and the aqueous layer was extracted with ethyl acetate (3 × 50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield *alcohol* **172** (0.90 g, 100%) as a pale yellow oil. This was used without further purification. $[\alpha]_D^{24}$ +22.4 (*c* 1.43, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3360 (OH), 2971, 2871, 1453, 1374, 1076, 972, 734, 697; δ_H(400 MHz) 1.02 (3H, d, *J* 7.0, 4-CH_3), 1.11 (3H, d, *J* 6.0, 6-H_3), 2.19 (1H, br s, OH), 2.39 (1H, m, 4-H), 3.42 (1H, app. quint., *J* 6.0, 5-H), 4.03 (2H, d, *J* 4.5, 1-H_2), 4.44 (1H, d, *J* 11.5, C*H*HPh), 4.56 (1H, d, *J* 11.5, CH*H*Ph), 5.56-5.68 (2H, m, 2-H and 3-H), 7.20-7.37 (5H, m, 5 × Ar-H); δ_C(100 MHz) 15.3 (4-CH_3), 16.2 (C-6), 41.2 (C-4), 63.5 (C-1), 70.6 (*C*H_2Ph), 78.2 (C-5), 127.3 (Ar-C), 127.5 (2 × Ar-C), 128.2 (2 × Ar-C), 129.2 and 134.7 (C-2 and C-3), 138.9 (Ar-C_{ipso}); *m/z* (ESI) 243 (MNa^+, 100%) and 203 (9); Found (ESI): 243.1361 (MNa^+), ($C_{14}H_{20}O_2Na$ requires 243.1356).

(2*S*,3*R*,4*S*,6*S*)-2-((1′*E*,3′*R*,4′*S*)-4′-(Benzyloxy)-3′-methylpent-1′-enyl)-3-methyl-1,8-dioxa-bicyclo[1.2.3]octane 174 and (2*S*,3*R*,4*S*,6*S*)-6-Benzyloxymethyl)-2-[(1*E*, 3*R*,4*S*)-4-(benzyloxy)-3-methylpent-1-enyl]-4-hydroxy-3-methyltetrahydropyran 175

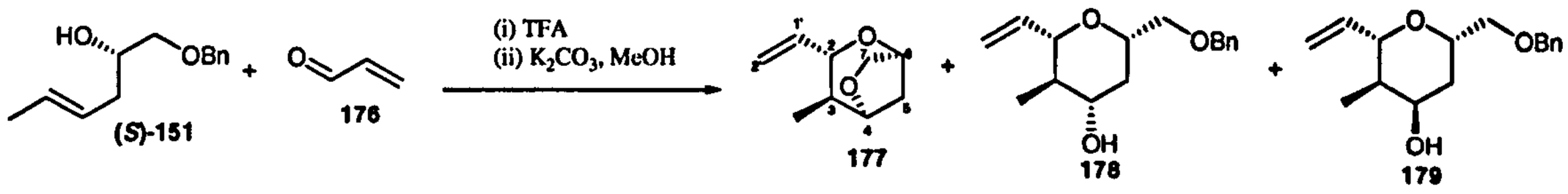


A solution of alcohol **172** (0.69 g, 3.13 mmol) in dry DCM (10 mL) was added dropwise to Dess-Martin periodinane (15% w/w in DCM, 9.0 mL, 4.34 mmol) in dry DCM (7.5 mL) at room temperature under an atmosphere of nitrogen. The reaction mixture was stirred for 1 hour and was then added to sodium hydroxide (1.0 M, 25

mL). Water (20 mL) was added to dissolve the resulting precipitate and the solution was then extracted with diethyl ether (3 × 50 mL). The extracts were washed with water (25 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield yellow oil. The aldehyde produced (0.66 g, 3.02 mmol) was added to a solution of alcohol (*S*)-**151** (0.94 g, 6.26 mmol) in dry DCM (20 mL) under an atmosphere of nitrogen. Trifluoroacetic acid (16.8 mL, 219.10 mmol) was added slowly and the mixture was stirred for 4 hours. The reaction was quenched with saturated aqueous sodium hydrogen carbonate solution (50 mL) and the pH was adjusted to above 7 by addition of triethylamine. The layers were separated and the aqueous phase was extracted with DCM (3 × 50 mL). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield a yellow oil. Potassium carbonate (4.33 g, 31.30 mmol) and methanol (50 mL) were added and the mixture was stirred for 1 hour. Volatiles were removed *in vacuo* and then water (100 mL) and DCM (100 mL) were added. The layers were separated and the aqueous phase was extracted with DCM (3 × 100 mL). The combined extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to give an orange/brown oil. Purification by flash column chromatography (SiO_2, 2.5-5% ethyl acetate/petroleum ether 40-60 °C) furnished the *bicyclic ether* **174** (0.30 g, 31%) as a colourless oil and *tetrahydropyran* **175** (0.074 g, 6%) as a yellow oil. *Bicyclic ether* **174**: $[\alpha]_D^{24}$ +43.5 (*c* 4.05, $CHCl_3$); ν_{max}(neat)/cm^{-1} 2966, 2933, 2874, 1454, 1373, 1204, 1092, 1062, 1002, 972, 870, 734, 697; δ_H(400 MHz) 0.84 (3H, d, *J* 7.0, 3-CH_3), 1.05 (3H, d, *J* 7.0, 3′-CH_3), 1.12 (3H, d, *J* 6.0, 5′-H_3), 1.38 (1H, dq, *J* 9.5, 7.0, 3-H), 1.82 (1H, ddd, *J* 11.5, 6.5, 3.0, 5-*H*H), 1.90 (1H, d, *J* 11.5, 5-H*H*), 2.40, (1H, m, 3′-H), 3.43 (1H, m, 4′-H), 3.80 (2H, m, 7-*H*H and 2-H), 4.08 (1H, d, *J* 10.0, 7-H*H*), 4.18 (1H, d, *J* 6.5, 4-H), 4.46 (1H, d, *J* 11.5, OCH_2Ph), 4.51 (1H, m, 6-H), 4.57 (1H, d, *J* 11.5, OCH_2Ph), 5.39 (1H, dd, *J* 15.5, 8.0, 1′-H), 5.71 (1H, dd, *J* 15.5, 8.0, 2′-H), 7.13-7.51 (5H, m, 5 × Ar-H); δ_C(100 MHz) 15.0 (3-CH_3), 15.5 and 16.4 (3′-CH_3 and C-5′), 38.2 (C-5), 41.5 and 41.7 (C-3 and C-3′), 70.6 and 71.1 (C-1″ and O*C*H_2Ph), 75.1 (C-6), 78.3 and 78.6 and 79.0 (C-2, C-4 and C-4′), 127.3 (Ar-C), 127.5 (2 × Ar-C), 128.2 (2 × Ar-C), 129.4 (C-1′), 137.3 (C-2′), 139.0 (Ar-C_{ipso}); *m/z* (ESI) 339 (MNa^+, 100%), 334 (35), 317 (3), 271 (33), 266 (3), 215 (3) and 113 (5); Found (ESI): 339.1934 (MNa^+), ($C_{20}H_{28}O_3Na$ requires 339.1931). *Tetrahydropyran* **175**: $[\alpha]_D^{22}$ +12.17 (*c* 4.93, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3417 (OH), 2965, 2928, 2869, 1496, 1453, 1095, 1060, 908, 734, 697;

δ_H(400 MHz) 0.90 (3H, d, *J* 6.5, 3-CH_3), 1.04 (3H, d, *J* 7.0, 3′-CH_3), 1.12 (3H, d, *J* 6.5, 5′-H_3), 1.25 (1H, m, 3-H), 1.38 (1H, app. q, *J* 12.5, 5-*H*H), 1.97 (1H, ddd, *J* 12.5, 5.0, 2.0, 5-H*H*), 2.48 (1H, m, 3′-H), 3.25-3.75 (6H, m, 2-H, 4-H, 4′-H, 6-H and 7-H_2), 4.42-4.60 (4H, m, 2 × OCH_2Ph), 5.43 (1H, ddd, *J* 15.5, 8.0, 1.0, 1′-H), 5.70 (1H, dd, *J* 15.5, 7.0, 2′-H), 7.23-7.36 (10H, m, 10 × Ar-H); δ_C(100 MHz) 13.3 (3′-CH_3), 14.5 (3-CH_3), 16.0 (C-5′), 37.7 (C-5), 40.8 (C-3′), 43.7 (C-3), 70.5 (OCH_2Ph), 73.0 and 73.3 (OCH_2Ph and C-7), 73.3 and 74.5 and 78.0 and 83.1 (C-2, C-4, C-4′ and C-6), 127.3 (Ar-C), 127.5 (2 × Ar-C), 127.6 (Ar-C), 127.7 (2 × Ar-C), 128.2 (2 × Ar-C), 128.3 (2 × Ar-C), 128.8 (C-1′), 136.8 (C-2′), 138.2 (Ar-C_{ipso}), 139.0 (Ar-C_{ipso}); *m/z* (ESI) 447 (MNa^+, 100%), 431 (53), 395 (14), 379 (46), 363 (19) and 149 (10); Found (ESI): 447.2495 (MNa^+), ($C_{27}H_{36}O_4Na$ requires 447.2506).

(2*S*,3*R*,4*S*,6*S*)-2-vinyl)-3-methyl-1,8-dioxa-bicyclo[1.2.3]octane 177, (2*S*,3*R*,4*S*,6*S*)-6-((Benzyloxy)methyl)-4-hydroxy-3-methyl-2-vinyl-tetrahydropyran 178 and (2*S*,3*R*,4*R*,6*S*)-6-((Benzyloxy)methyl)-4-hydroxy-3-methyl-2-vinyl tetrahydropyran 179

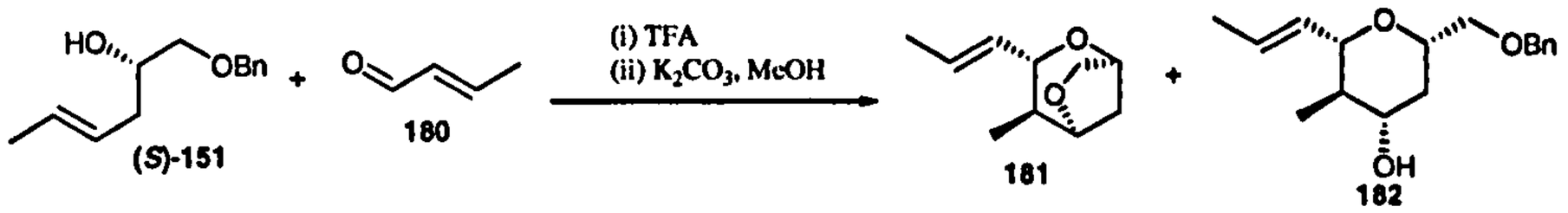


Alcohol (*S*)-**151** (0.50 g, 2.43 mmol) and propenal **176** (0.66 mL, 9.70 mmol) were dissolved in dry DCM (25 mL) under an atmosphere of nitrogen. Trifluoroacetic acid (7.4 mL, 97.00 mmol) was added slowly and the mixture was stirred for 4 hours. The reaction was quenched with saturated aqueous sodium hydrogen carbonate solution (60 mL) and the pH was adjusted to above 7 by addition of triethylamine. The layers were separated and the aqueous phase was extracted with DCM (3 × 50 mL). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield yellow oil. Potassium carbonate (2.24 g, 16.17 mmol) and methanol (25 mL) were added and the mixture was stirred for 1 hour. Volatiles were removed *in vacuo* and then water (50 mL) and DCM (50 mL) were added. The layers were separated and the aqueous phase was extracted with DCM (3 × 50 mL). The combined extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to give a yellow oil. Purification by flash column chromatography (SiO_2, 1-10% ethyl acetate/petroleum ether 40-60 °C) gave *bicyclic ether* **177** (0.050 g, 14%) as a colourless oil, *tetrahydropyran* **178** (0.029 g, 5%) as a yellow oil and a second

tetrahydropyran **179** (0.009 g, 2%) also as a yellow oil. *Bicyclic ether* **177**: $[\alpha]_D^{22}$ +49.0 (*c* 2.49, $CHCl_3$); ν_{max}(neat)/cm^{-1} 2965, 2935, 2877, 1740, 1455, 1376, 1222, 1205, 1133, 1062, 870, 699; δ_H(400 MHz) 0.89 (3H, d, *J* 7.0, 3-CH_3), 1.42 (1H, dq, *J* 9.5, 7.0, 3-H), 1.83 (1H, ddd, *J* 11.5, 6.0, 3.0, 5-*H*H), 1.92 (1H, dt, *J* 11.5, 1.5, 5-H*H*), 3.81-3.86 (2H, m, 2-H and 7-*H*H), 4.10 (1H, dd, *J* 10.0, 1.0, 7-H*H*), 4.21 (1H, d, *J* 6.0, 4-H), 4.54 (1H, app. dt, *J*, 3.0, 1.5, 6-H), 5.19 (1H, ddd, *J* 10.5, 1.5, 1.0, 2′-*H*H), 5.28 (1H, ddd, *J* 17.0, 1.5, 1.0, 2′-H*H*), 5.78 (1H, ddd, *J* 17.0, 10.5, 7.5, 1′-H); δ_C(100 MHz) 14.8 (3-CH_3), 38.1 (C-5), 41.2 (C-3), 71.1 (C-7), 75.1 (C-6), 78.6 and 79.0 (C-2 and C-4), 117.5 (C-2′), 137.3 (C-1′); *m/z* (EI) 154 (M^+, 4%), 139 (3), 123 (4), 110 (10), 85 (62), 83 (100), 69 (37) and 55 (24); Mass ion could not be detected at high resolution and so an accurate mass has not been included. *Tetrahydropyran* **178**: $[\alpha]_D^{22}$ +2.4 (*c* 1.69, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3403 (OH), 2859, 1721, 1454, 1365, 1101, 1046, 735, 697; δ_H(400 MHz) 0.95 (3H, d, *J* 7.0, CH_3), 1.29 (1H, m, 3-H), 1.41 (1H, app. q, *J* 12.5, 5-*H*H), 1.68 (1H, br s, OH), 2.01 (1H, ddd, *J* 12.5, 5.0, 2.0, 5-H*H*), 3.39 (1H, m, 2-H), 3.45 (1H, m, 4-H), 3.47 (1H, dd, *J* 10.0, 5.0, 7-*H*H), 3.57 (1H, dd, *J* 10.0, 5.5, 7-H*H*), 3.66 (1H, m, 6-H), 4.57 (2H, d, *J* 2.0, CH_2Ph), 5.22 (1H, dd, *J* 10.5, 2.0, 2′-*H*H), 5.27 (1H, dt, *J* 17.0, 1.0, 2′-H*H*), 5.81 (1H, ddd, *J* 17.0, 10.5, 7.5, 1′-H), 7.27-7.37 (5H, m, 5 × Ar-H); δ_C(100 MHz) 13.1 (3-CH_3), 37.8 (C-5), 43.5 (C-3), 73.0 (C-7 or *C*H_2Ph), 73.4 (two coincident signals, C-2 and either C-7 or *C*H_2Ph), 74.7 (C-6), 83.1 (C-4), 117.9 (C-2′), 127.6 (Ar-C), 127.7 (2 × Ar-C), 128.4 (2 × Ar-C), 136.8 (C-1′), 138.3 (Ar-C_{ipso}); *m/z* (CI) 263 (MH^+, 18%), 245 (21), 189 (18), 171 (21), 159 (15), 99 (17), 91 (100) and 87 (18); Found (CI): 263.1641 (MH^+), ($C_{16}H_{23}O_3$ requires 263.1647). *Tetrahydropyran* **179**: $[\alpha]_D^{23}$ -6.5 (*c* 0.62, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3408 (OH), 2927, 1722, 1454, 1099, 1040, 989, 925, 736, 697; δ_H(400 MHz) 0.96 (3H, d, *J* 6.5, 3-CH_3), 1.41 (1H, m, 3-H), 1.70 (1H, ddd, *J* 13.0, 10.0, 6.0, 5-*H*H); 2.01 (1H, ddd, *J* 13.0, 4.5, 3.0, 5-H*H*), 3.55 (1H, dd, *J* 10.0, 5.5, 7-*H*H), 3.62 (1H, m, 4-H), 3.66 (1H, dd, *J* 10.0, 6.5, 7-HH), 3.75 (1H, app. t, *J* 8.0, 2-H), 4.26 (1H, m, 6-H), 4.58 (2H, s, CH_2Ph), 5.19-5.28 (2H, m, 2′-H_2), 5.83 (1H, ddd, *J* 17.0, 10.5, 7.0, 1′-H), 7.27-7.38 (5H, m, 5 × Ar-H); δ_C(100 MHz) 13.9 (3-CH_3), 34.6 (C-5), 42.9 (C-3), 70.0 (C-4 or C-6), 70.6 (C-7), 70.9 (C-4 or C-6), 73.2 (*C*H_2Ph), 77.8 (C-2), 117.5 (C-2′), 127.6 (2 × Ar-C), 127.7 (Ar-C), 128.4 (2 × Ar-C), 137.5 (C-1′), 138.2 (Ar-C_{ipso}); *m/z* (CI) 263 (MH^+, 19%), 261 (20), 245 (30), 209 (22), 203 (53), 197 (27),

91 (100), 87 (29), 85 (65) and 83 (94); Found (ESI): 263.1639 (MH^+), ($C_{16}H_{23}O_3$ requires 263.1647).

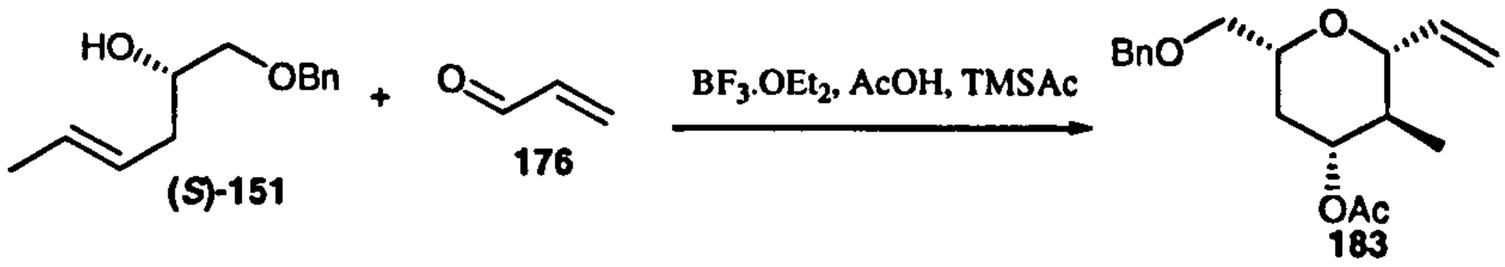
(2*S*,3*R*,4*S*,6*S*)-2-Crotyl)-3-methyl-1,8-dioxa-bicyclo[1.2.3]octane 181 and (2*S*,3*R*,4*S*,6*S*)-6-((Benzyloxy)methyl)-2-crotyl-4-hydroxy-3-methyl-tetrahydropyran 182[99]



Alcohol (*S*)-**151** (0.50 g, 2.43 mmol) and but-2-enal **180** (0.80 mL, 9.70 mmol) were dissolved in dry DCM (25 mL) under an atmosphere of nitrogen. Trifluoroacetic acid (7.4 mL, 97.00 mmol) was added slowly and the mixture was stirred for 2.5 hours. The reaction was quenched with saturated aqueous sodium hydrogen carbonate solution (50 mL) and the pH was adjusted to above 7 with triethylamine. The layers were separated and the aqueous phase was extracted with DCM (3 × 50 mL). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield yellow oil. Potassium carbonate (2.24 g, 16.17 mmol) and methanol (25 mL) were added and the mixture was stirred for 1 hour. Volatiles were removed *in vacuo* and then water (50 mL) and DCM (50 mL) were added. The layers were separated and the aqueous phase was extracted with DCM (3 × 50 mL). The combined extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to give a yellow oil. Purification by flash column chromatography (SiO_2, 2.5-30% ethyl acetate/petroleum ether 40-60 °C) gave *bicyclic ether* **181** (0.050 g, 59%) as a colourless oil and tetrahydropyran **182** (0.029 g, 7%) as a yellow oil. Although a known compound, tetrahydropyran **182** has not been previously characterised.[99] *Bicyclic ether* **181**: $[\alpha]_D^{24}$ +43.8 (*c* 1.30, $CHCl_3$); ν_{max}(neat)/cm^{-1} 2964, 2936, 2876, 1677, 1454, 1376, 1290, 1221, 1206, 1135, 1061, 1001, 964, 869, 763; δ_H(400 MHz) 0.84 (3H, d, *J* 6.5, 3-CH_3), 1.39 (1H, dq, *J* 9.5, 6.5, 3-H), 1.70 (3H, dd, *J* 6.5, 1.5, 3′-H_3), 1.80 (1H, ddd, *J* 11.5, 6.5, 3.0, 5-*H*H), 1.89 (1H, dt, *J* 11.5, 1.0, 5-H*H*), 3.76 (1H, m, 2-H), 3.80 (1H, dd, *J* 10.0, 3.0, 7-*H*H), 4.07 (1H, dd, *J* 10.0, 1.0, 7-H*H*), 4.18 (1H, d, *J* 6.5, 4-H), 4.50 (1H, m, 6-H), 5.40 (1H, ddq, *J* 15.0, 8.0, 1.5, 1′-H), 5.73 (1H, dqd, *J* 15.0, 6.5, 1.0, 2′-H); δ_C(100 MHz) 14.9 (3-CH_3), 17.7 (C-3′), 38.1 (C-5), 41.4 (C-3), 71.1 (C-7), 75.0 (C-6), 78.3 (C-2), 78.9 (C-4), 129.8 (C-2′), 130.4 (C-1′); *m/z* (ESI) 168 (M^+, 23%), 153 (22), 149 (18), 91 (32), 85 (62), 83 (100),

69 (11) and 55 (10); Found (EI): 168.1146 (M^+), ($C_{10}H_{16}O_2$ requires 168.1150). Tetrahydropyran **182**: $[\alpha]_D^{24}$ +2.6 (*c* 2.30, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3401 (OH), 2916, 2857, 1453, 1365, 1037, 965, 933, 733, 697; δ_H(400 MHz) 0.93 (3H, d, *J* 6.5, 3-CH_3), 1.29 (1H, app. tq, *J* 6.5, 3.0, 3-H), 1.40 (1H, app. q, *J* 12.0, 5-*H*H), 1.72 (3H, dd, *J* 6.5, 1.5, 3′-H_3), 1.77 (1H, br s, OH), 2.00 (1H, ddd, *J* 12.0, 4.5, 2.0, 5-H*H*), 3.34-3.43 (2H, m, 2-H and 4-H), 3.47 (1H, dd, *J* 10.0, 4.5, 7-*H*H), 3.57 (1H, dd, *J* 10.0, 5.5, 7-H*H*), 3.64 (1H, m, 6-H), 4.55 (1H, d, *J* 12.0, C*H*HPh), 4.59 (1H, d, *J* 12.0, CH*H*Ph), 5.44 (1H, ddq, *J* 15.0, 8.0, 1.5, 1′-H), 5.72 (1H, dqd, *J* 15.5, 6.5, 1.0, 2′-H), 7.27-7.36 (5H, m, 5 × Ar-H); δ_C(100 MHz) 13.2 (3-CH_3), 17.8 (C-3′), 37.7 (C-5), 43.6 (C-3), 72.9 and 73.3 (C-7 and CH_2Ph), 73.4 (C-2), 74.4 (C-6), 82.9 (C-4), 127.5 (C-2′), 127.7 (2 × Ar-C), 128.3 (2 × Ar-C), 129.9 (Ar-C), 130.1 (C-1′), 138.2 (Ar-C_{ipso}); *m/z* (ESI) 299 (MNa^+, 100%); Found (ESI): 299.1608 (MNa^+), ($C_{17}H_{24}O_3Na$ requires 299.1618).

(2*S*,3*R*,4*S*,6*S*)-4-Acetoxy-6-((benzyloxy)methyl)-3-methyl-2-vinyl tetrahydropyran 183



A stirred solution of homoallylic alcohol (*S*)-**151** (0.30 g, 1.45 mmol), propenal **176** (0.12 mL, 1.74 mmol), acetic acid (0.42 mL, 7.25 mmol) and trimethylsilyl acetate (0.87 mL, 5.80 mmol) in dry hexane (12 mL) was treated dropwise with boron trifluoride diethyletherate (0.37 mL, 2.90 mmol) under an atmosphere of nitrogen. A brown precipitate formed. The solution was stirred vigorously for 23 hours and was then quenched with saturated aqueous sodium hydrogen carbonate solution (25 mL). The mixture was extracted with DCM (3 × 50 mL), washed with water (10 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield a pale yellow oil. Purification by flash column chromatography (SiO_2, 10% ethyl acetate/petroleum ether 40-60 °C) gave *tetrahydropyran* **183** (0.60 g, 14%) as a pale yellow oil. $[\alpha]_D^{23}$ +19.11 (*c* 3.14, $CHCl_3$); ν_{max}(neat)/cm^{-1} 2968, 2932, 2860, 1730 (C=O), 1454, 1363, 1236, 1104, 1027, 736 and 698; δ_H(400 MHz) 0.84 (3H, d, *J* 6.5, 3-CH_3), 1.46 (1H, app. q, *J* 12.0, 5-*H*H), 1.55 (1H, m, 3-H), 2.06 (4H, m, 5-H*H* and $COCH_3$), 3.40-3.63 (3H, m, 2-H and 7-H_2), 3.69 (1H, m, 6-H), 4.51-4.60 (2H, m, OCH_2Ph), 4.67 (1H,

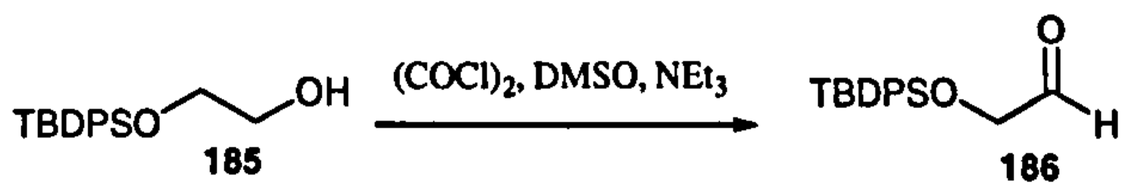
app. td, *J* 11.0, 4.5, 4-H), 5.24 (1H, dm, *J* 10.5, 2'-*H*H), 5.29 (1H, dm, *J* 17.0, 2'-H*H*), 5.80 (1H, ddd, *J* 17.0, 10.5, 7.5, 1'-H), 7.27-7.37 (5H, m, 5 × Ar-H); δ_C(100 MHz) 13.1 (3-CH_3), 21.2 (CH_3CO), 34.1 (C-5), 40.3 (C-3), 72.7 and 73.4 (C-7 and CH_2Ph), 74.4 (C-4), 75.2 (C-6), 83.1 (C-2), 118.3 (C-2'), 127.6 (Ar-C), 127.7 (2 × Ar-C), 128.4 (2 × Ar-C), 136.4 (Ar-C_{ipso}), 138.2 (C-1'), 170.7 (CO); *m/z* (CI) 305 (MH^+, 16%), 295 (68), 245 (46), 189 (68), 181 (56), 171 (88), 159 (60) and 91 (100); Found (CI): 305.1747 (MH^+), ($C_{18}H_{25}O_4Na$ requires 305.1753).

2-(*tert*-Butyldiphenylsilyloxy)ethanol 185[190]

167 185

n-Butyllithium (2.5 M in hexanes, 18.50 mL, 46.25 mmol) was added dropwise to a solution of ethane-1, 2-diol **167** (2.56 mL, 46.00 mmol) in dry THF (95 mL) at -78 °C under an atmosphere of nitrogen. After 15 minutes, *tert*-butylchlorodiphenylsilane (11.96 mL, 46.00 mmol) was added and the mixture was stirred at -78 °C for 15 minutes and then allowed to warm to room temperature. The solution was refluxed for 24 hours and then the solvent was removed *in vacuo* to give a pale yellow oil interspersed with white solid. Purification by flash column chromatography (SiO_2, 5-50% ethyl acetate/petroleum ether 40-60 °C) furnished alcohol **185** (12.01 g, 87%) as a pale yellow oil. δ_H(400 MHz) 1.07 (9H, s, $SiC(CH_3)_3$), 2.16 (1H, t, *J* 6.0, OH), 3.68 and 3.76 (each 2H, each m, 1-H_2 and 2-H_2), 7.36-7.46 (6H, m, 6 × Ar-H), 7.65-7.69 (4H, m, 4 × Ar-H); δ_C(100 MHz) 19.2 ($SiC(CH_3)_3$), 26.9 ($SiC(CH_3)_3$), 63.7 and 65.0 (C-1 and C-2), 127.8 (4 × Ar-C), 129.8 (2 × Ar-C), 133.3 (2 × Ar-C_{ipso}), 135.5 (4 × Ar-C).

2-(*tert*-Butyldiphenylsilyloxy)ethanal 186[190]

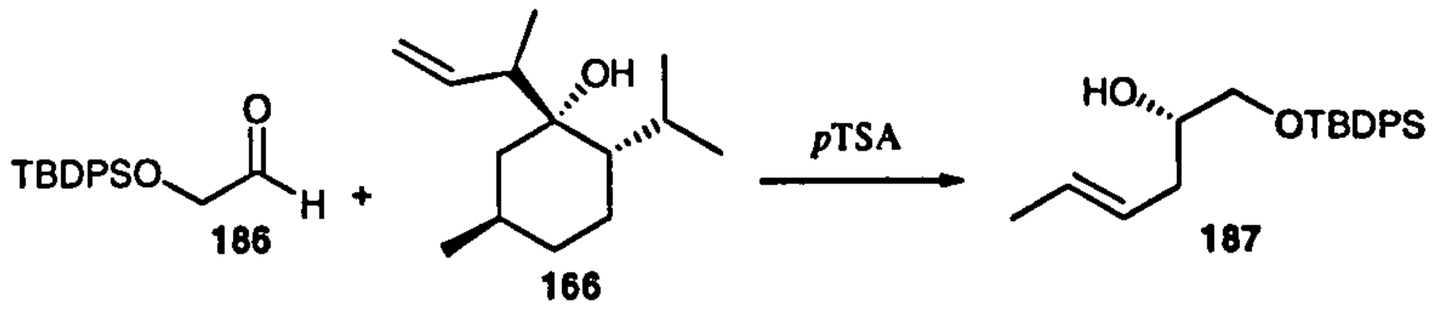


185 186

Dimethylsulfoxide (3.7 mL, 51.90 mmol) was added slowly to a solution of oxalyl chloride (2.3 mL, 25.96 mmol) in dry DCM (40 mL) at -78 °C under an atmosphere of nitrogen. The mixture was stirred for 10 minutes and then a solution of alcohol **185** (6.00 g, 19.96 mmol) in dry DCM (16 mL) was added dropwise. The reaction mixture was stirred for 20 minutes and then triethylamine (freshly distilled over calcium hydride, 16.6 mL, 119.76 mmol) was added. After a further 20 minutes the reaction

was allowed to warm to room temperature and stirred for 1 hour. Saturated aqueous ammonium chloride solution (80 mL) was added and the layers were separated. The aqueous phase was extracted with DCM (3 × 100 mL) and the combined organic extracts were washed with hydrochloric acid (0.5 M, 100 mL) and brine (50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield a yellow oil. Purification by flash column chromatography (SiO_2, 10% ethyl acetate/petroleum ether 40-60 °C) furnished aldehyde **186** (3.00 g, 50%) as a colourless oil. δ_H(400 MHz) 1.10 (9H, s, $SiC(CH_3)_3$), 4.21 (2H, s, 2-H_2), 7.37-7.47 (6H, m, 6 × Ar-H), 7.63-7.70 (4H, m, 4 × Ar-H), 9.72 (1H, s, 1-H); δ_C(100 MHz) 19.3 ($SiC(CH_3)_3$), 26.7 ($SiC(CH_3)_3$), 70.0 (C-2), 128.0 (4 × Ar-C), 130.1 (2 × Ar-C), 132.5 (2 × Ar-C_{ipso}), 135.5 (4 × Ar-C), 201.7 (C-1).

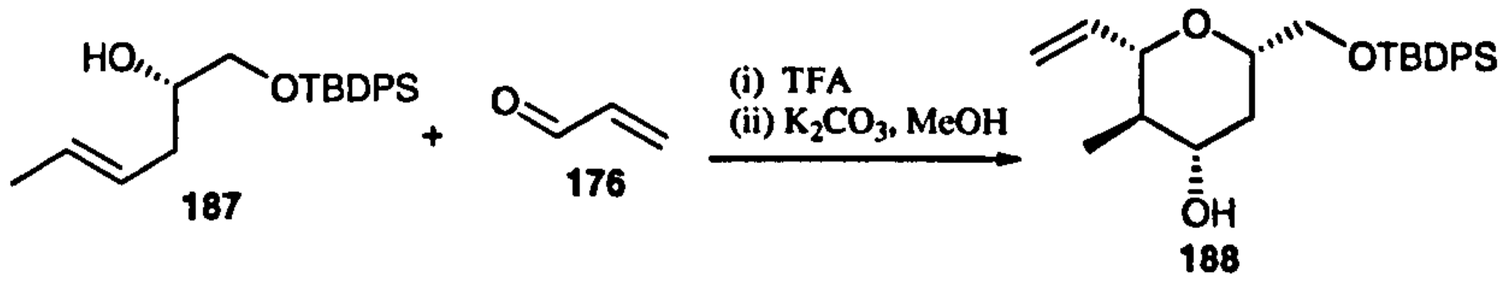
(2*S*,4*E*)-1-(*tert*-Butyldiphenylsilyloxy)hex-4-en-2-ol 187



p-Toluenesulfonic acid monohydrate (0.18 g, 0.95 mmol) was added to a solution of aldehyde **186** (2.92 g, 9.73 mmol) and alcohol **166** (4.00 g, 19.01 mmol) in dry DCM (100 mL) at room temperature. The reaction was stirred for 3 days and was then quenched with triethylamine (1.00 mL) and saturated aqueous ammonium chloride solution (100 mL). After 15 minutes of vigorous stirring the layers were separated and the aqueous layer was extracted with DCM (4 × 75 mL). The combined extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield an orange oil. Purification by flash column chromatography (SiO_2, 1% ethyl acetate/petroleum ether 40-60 °C) yielded *homoallylic alcohol* **187** (2.61g, 76%) as a pale yellow oil. $[\alpha]_D^{23}$ -2.86 (*c* 1.40, $CHCl_3$), ν_{max}(neat)/cm^{-1} 3421 (OH), 2931, 2892, 2857, 1428 (C=C), 1111, 699; δ_H(400 MHz) 1.07 (9H, s, $SiC(CH_3)_3$), 1.63 (3H, dq, *J* 6.5, 1.5, 6-H_3), 2.14-2.19 (2H, m, 3-*H*H and OH), 2.41 (1H, m, 3-H*H*), 3.54 (1H, dd, *J* 10.0, 7.0, 1-*H*H), 3.65 (1H, dd, *J* 10.0, 4.0, 1-H*H*), 3.72 (1H, m, 2-H), 5.38 (1H, dtq, *J* 15.0, 7.0, 1.5, 5-H), 5.49 (2H, dqt, *J* 15.0, 6.5, 1.0, 4-H), 7.33-7.46 (6H, m, 6 × Ar-H), 7.60-7.73 (4H, m, 4 × Ar-H); δ_C(100 MHz) 18.0 (C-6), 19.3 ($SiC(CH_3)_3$), 26.9 ($SiC(CH_3)_3$), 36.4 (C-3), 67.3 (C-1), 71.7 (C-2), 126.7 (C-4 or C-5), 127.8 (4 × Ar-C), 129.8 (2 × Ar-C), 133.3 (2 × Ar-C_{ipso}), 134.8 (C-4 or C-5), 135.6 (4 × Ar-C); *m/z* (ESI) 377

(MNa$^+$, 100%), 277 (9), 244 (15), 192 (11) and 125 (9); Found (ESI): 377.1915 (MNa$^+$), ($C_{22}H_{30}O_2SiNa$ requires 377.1907).

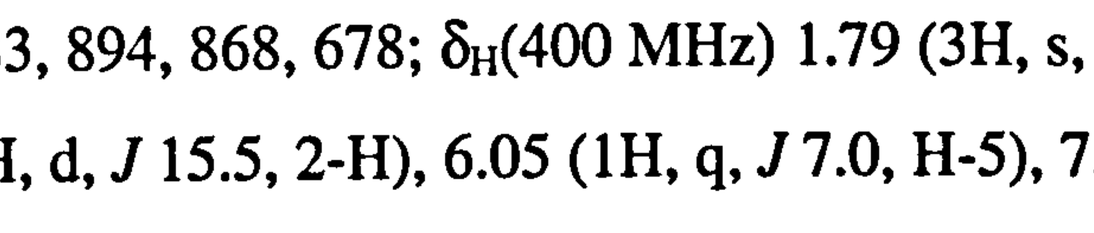
(2*S*,3*R*,4*S*,6*S*)-6-((*tert*-Butyldiphenylsilyloxy)methyl)-4-hydroxy-3-methyl-2-vinyl-tetrahydropyran 188



Alcohol **187** (0.30 g, 0.85 mmol) and propenal **176** (0.58 mL, 8.50 mmol) in dry DCM (8.5 mL) at 0 °C under an atmosphere of nitrogen. Trifluoroacetic acid (0.65 mL, 8.50 mmol) was added slowly and the reaction was stirred at room temperature for 5.5 hours. The reaction was quenched with saturated aqueous sodium hydrogen carbonate solution (20 mL) and the pH was adjusted to above 7 by addition of triethylamine. The layers were separated and the aqueous phase was extracted with DCM (3 × 50 mL). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield yellow oil. Potassium carbonate (0.59 g, 4.25 mmol) and methanol (6 mL) were added and the mixture was stirred for 2 hours. Volatiles were removed *in vacuo* and then water (50 mL) and DCM (50 mL) were added. The layers were separated and the aqueous phase was extracted with DCM (3 × 50 mL). The combined extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to give a yellow oil. Purification by flash column chromatography (SiO_2, 5-10% ethyl acetate/petroleum ether 40-60 °C) gave *tetrahydropyran* **188** (0.180 g, 52%). $[\alpha]_D^{23}$ -4.11 (*c* 4.87, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3393 (OH), 2930, 2857, 1428, 1111, 1048, 823, 739, 700; δ_H(400 MHz) 0.95 (3H, d, *J* 6.5, 3-CH_3), 1.05 (9H, s, SiC(CH_3)$_3$), 1.27 (1H, m, 3-H), 1.37 (1H, app. q, *J* 12.0, 5-*H*H), 1.72 (1H, br s, OH), 2.15 (1H, ddd, *J* 12.0, 4.5, 1.5, 5-H*H*), 3.30-3.45 (2H, m, 2-H and 4-H), 3.50-3.63 (2H, m, 6-H and 7-*H*H), 3.79 (1H, m, 7-H*H*), 5.19 (1H, dd, *J* 10.0, 1.5, 2′-*H*H), 5.26 (1H, ddd, *J* 17.5, 1.5, 1.0, 2′-H*H*), 5.77 (1H, ddd, *J* 17.5, 10.0, 7.5, 1′-H), 7.33-7.43 (6H, m, 6 × Ar-H), 7.65-7.70 (4H, m, 4 × Ar-H); δ_C(100 MHz) 13.1 (3-CH_3), 19.3 (Si*C*(CH_3)$_3$), 26.9 (SiC(*C*H_3)$_3$), 37.8 (C-5), 43.7 (C-3), 66.7 (C-7), 73.5 (C-2 or C-4), 75.8 (C-6), 82.9 (C-2 or C-4), 117.6 (C-2′), 127.6 (4 × Ar-C), 129.6 (2 × Ar-C), 133.6 (2 × Ar-C_{ipso}), 135.6 (4 × Ar-C), 136.9 (C-1′); *m/z* (ESI) 433

(MNa$^+$, 100%), 397 (24), 353 (10), 192 (76) and 167 (12); Found (ESI): 433.2179 (MNa$^+$), ($C_{25}H_{34}O_3SiNa$ requires 433.2169).

(2*E*,4*E*)-4-Methylhexa-2,4-dienoic acid 193[191,192]

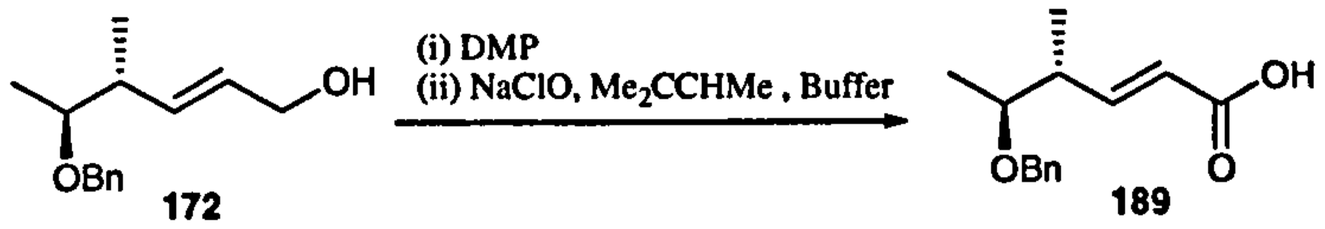


171 193

Ester **171** (0.17 g, 0.65 mmol) in ethanol (1 mL) and aqueous sodium hydroxide solution (2.0 M, 10 mL) was heated to reflux for 16 hours. The reaction was allowed to cool to room temperature and was then diluted with water (50 mL). The pH of the solution was adjusted to 2 with hydrochloric acid (2.0 M). The solution was extracted with ethyl acetate (3 × 50 mL) and the organic extracts were dried over sodium sulfate, filtered and concentrated *in vacuo* to give acid **193** (0.12 g, 82%) as colourless needles. m.p. 94 °C, lit.[191] 94-95 °C; ν_{max}(neat)/cm^{-1} 2987, 1688 (C=O), 1409, 1361, 1258, 1216, 1064, 983, 894, 868, 678; δ_H(400 MHz) 1.79 (3H, s, 4-CH_3), 1.84 (3H, d, *J* 7.0, 6-H_3), 5.80 (1H, d, *J* 15.5, 2-H), 6.05 (1H, q, *J* 7.0, H-5), 7.42 (1H, d, *J* 15.5, 3-H); δ_C(100 MHz) 11.8 (4-CH_3), 14.7 (C-6), 133.8 (C-2), 114.3 (C-4), 137.9 (C-5), 151.9 (C-3), 173.0 (C-1); *m/z* (CI) 127 (MH$^+$, 7%), 125 (47), 115 (100), 99 (28) and 97 (50); Found 127.0753 (CI): (MH$^+$), ($C_7H_{11}O_2$ requires 127.0759).

(2*E*,4*R*,5*S*)-5-Benzyloxy-4-methylhex-2-enoic acid 189

(i) *via* Dess-Martin Periodinane and Pinnick oxidation of alcohol 172

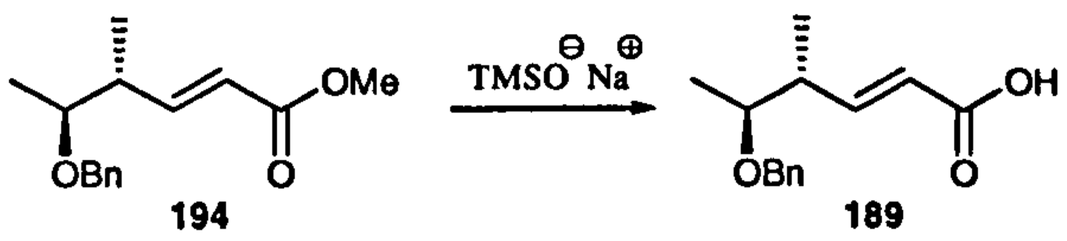


172 189

Alcohol **172** (0.50 g, 2.27 mmol) in dry DCM (13 mL) was added to a solution of Dess-Martin periodinane (15% w/t in DCM, 7.1 mL, 3.41 mmol) in DCM (13 mL) under an atmosphere of nitrogen. After stirring for 1 hour the reaction was deemed complete by T.L.C. and was diluted with ether (20 mL) prior to quenching with sodium hydroxide (1.0 M, 20 mL). The aqueous phase was extracted with ether (3 × 50 mL) and then the combined extracts were washed with water (2 × 30 mL), dried over magnesium sulfate and concentrated *in vacuo*. The resulting yellow oil was dissolved in *tert*-butanol (30 mL) and 2-methyl-but-2-ene (13 mL). A solution of sodium chlorite (2.44 g, 26.96 mmol) and sodium dihydrogen phosphate (2.04 g, 1.70

mmol) in water (15 mL) was added. The reaction was stirred for 2 hours and was then extracted with ethyl acetate (3 × 100 mL), dried over magnesium sulfate and concentrated *in vacuo* to give a colourless oil. Purification by flash column chromatography (SiO_2, 5% ethyl acetate/1% acetic acid/petroleum ether 40-60 °C) gave *acid* **189** (1.05 g, 88%) as a colourless oil. $[\alpha]_D^{22}$ +16.9 (*c* 4.02, $CHCl_3$); ν_{max}(neat)/cm^{-1} 2973, 2873, 1692 (C=O), 1649 (C=C), 1495, 1454, 1418, 1284, 1093, 1065, 984, 984, 924, 733, 696; δ_H(400 MHz) 1.09 (3H, d, *J* 7.0, 4-CH_3), 1.15 (3H, d, *J* 6.0, 6-H_3), 2.56 (1H, m, 4-H), 3.50 (1H, dq, *J* 6.0, 5.5, 5-H), 4.45 (1H, d, *J* 12.0, C*H*HPh), 4.59 (1H, d, *J* 12.0, CH*H*Ph), 5.85 (1H, dd, *J* 15.5, 1.5, 2-H), 7.11 (1H, dd, *J* 15.5, 7.5, 3-H), 7.24-7.35 (5H, m, 5 × Ar-H), 11.58 (1H, br s, COOH); δ_C(100 MHz) 14.6 (4-CH_3), 16.6 (C-6), 42.0 (C-4), 70.8 (CH_2Ph), 77.4 (C-5), 120.7 (C-2), 127.5 (Ar-C), 127.6 (2 × Ar-C), 128.4 (2 × Ar-C), 138.5 (Ar-C_{ipso}), 154.2 (C-3), 172.2 (C-1); *m/z* (ESI) 257 (MNa^+, 100%), 252 (14) and 115 (18); Found (ESI): 257.1147 (MNa^+), ($C_{14}H_{18}O_3Na$ requires 257.1148).

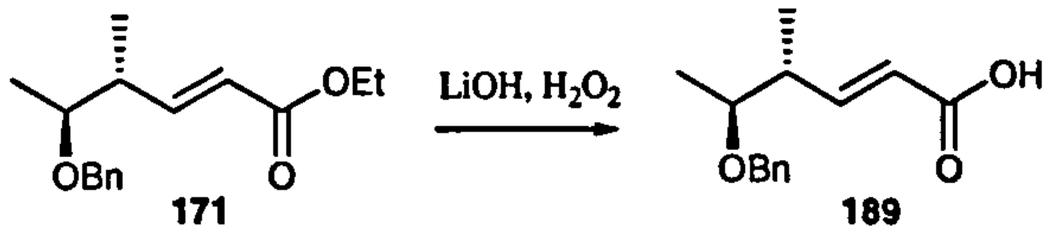
(ii) *via* hydrolysis of methyl ester 194[108]



194 **189**

Sodium trimethylsilanoate (1.0 M in DCM, 0.4 mL, 0.40 mmol) was added to a solution of methyl ester **194** (0.05 g, 0.20 mmol) in dry DCM (4 mL) under an atmosphere of nitrogen. The reaction was stirred overnight and then volatiles were removed *in vacuo* to give the sodium salt as a pale yellow solid. Purification by flash column chromatography (SiO_2, 20% ethyl acetate/1% acetic acid/petroleum ether 40-60 °C) gave *acid* **189** (0.037 g, 85%) as a colourless oil. Spectral data as above.

(iii) *via* hydrolysis of ethyl ester 171

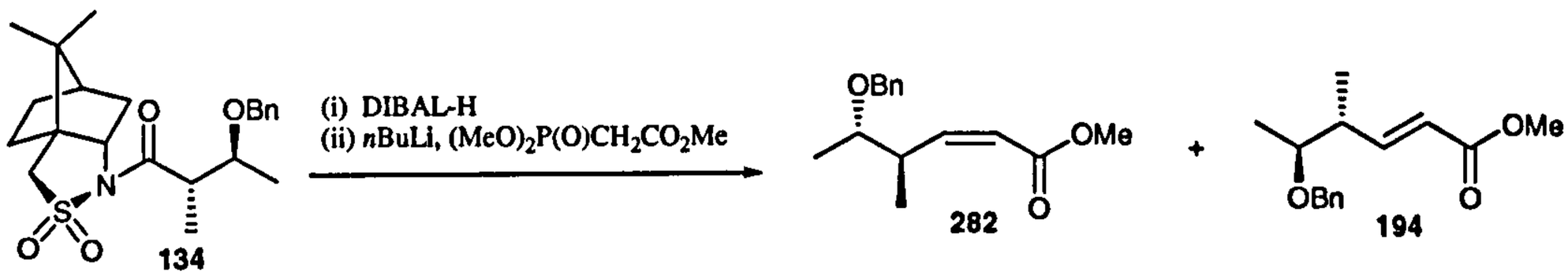


171 **189**

Lithium hydroxide (0.027 g, 1.14 mmol) and hydrogen peroxide (30% in water, 0.2 mL, 1.71 mmol) in THF (6 mL) and water (1.8 mL) was cooled to 0 °C. Ester **171** (0.15 g, 0.57 mmol) in THF (1 mL) was added dropwise and the reaction was allowed to warm to room temperature. After 1 week, the reaction was cooled to 0 °C and

quenched with sodium sulfite solution (20 mL). Solvents were removed *in vacuo* and then water (17 mL) was added. The pH of the solution was adjusted to 2 with hydrochloric acid (6.0 M) and the solution was then extracted with DCM (3 × 50 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo* to give a colourless oil. Purification by flash column chromatography (SiO_2, 5% ethyl acetate/petroleum ether 40-60 °C) furnished *acid* **189** (0.02 g, 15%) as a colourless oil. Spectral data as above.

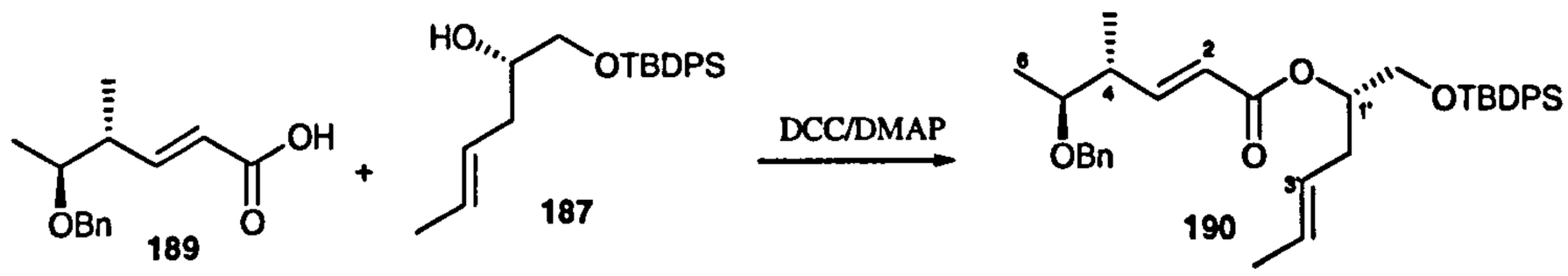
Methyl (2*Z*,4*R*,5*S*)-5-Benzyloxy-4-methylhex-2-enoate 282 and methyl (2*E*,4*R*,5*S*)-5-Benzyloxy-4-methylhex-2-enoate 194



Benzyl ether **134** (0.50 g, 1.23 mmol) was dissolved in dry DCM (10 mL) under an atmosphere of nitrogen and cooled to -78 °C. Diisobutylaluminium hydride (1.0 M in hexanes, 1.2 mL, 1.2 mmol) was added dropwise in 3 portions at 15 minute intervals. After 1.5 hours a further addition of diisobutylaluminium hydride (1.0 M in hexanes, 0.62 mL, 0.62 mmol) was required. After 2.5 hours the reaction was quenched with saturated aqueous potassium sodium L-tartrate tetrahydrate solution (20 mL). The reaction was warmed to room temperature and stirred vigorously overnight to break up the aluminium complex. The mixture was then extracted with DCM (3 × 50 mL) and the combined organic layers were washed with water (5 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* on a cool rotary evaporator. The resulting oil was triturated with petroleum ether 40-60 °C and filtered to remove the precipitated auxiliary. The filtrate was concentrated *in vacuo* to yield a colourless oil. This was then dissolved in dry THF (3 mL) and added dropwise to a solution of trimethyl phosphonoacetate (0.44 mL, 2.71 mmol) and *n*-butyllithium (2.5 M in hexanes, 1.1 mL, 2.71 mmol) in dry THF (15 mL) which had been stirred for 1 hour at 0 °C under an atmosphere of nitrogen. The resulting solution was stirred at room temperature for 20 hours and was then quenched with water (25 mL). The solution was extracted with ethyl acetate (3 × 50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to give a pale yellow oil. Purification by flash column chromatography (SiO_2, 2.5-5% ethyl acetate/petroleum ether 40-60 °C) furnished *(Z)*-

methyl ester **282** (0.049 g, 16%) then *(E)-methyl ester* **194** (0.073 g, 24%) both as a colourless oils. *(Z)-methyl ester* **282**: $[\alpha]_D^{20}$ -33.7 (*c* 2.52, $CHCl_3$); ν_{max}(neat)/cm^{-1} 2969, 2929, 2873, 1720 (C=O), 1644 (C=C), 1454, 1437, 1195, 1176, 825, 733, 696; δ_H(400 MHz) 1.08 (3H, d, *J* 7.0, 4-CH_3), 1.15 (3H, d, *J* 6.0, 6-H_3), 3.50 (1H, dq, *J* 6.0, 4.5, 5-H), 3.70 (3H, s, OCH_3), 3.75 (1H, m, 4-H), 4.49 (1H, d, *J* 11.5, C*H*HPh), 4.61 (1H, d, *J* 11.5, CH*H*Ph), 5.81 (1H, dd, *J* 11.5, 1.0, 2-H), 6.23 (1H, dd, *J* 11.5, 10.0, 3-H), 7.24-7.36 (5H, m, 5 × Ar-H); δ_C(100 MHz) 16.2 (4-CH_3), 17.0 (C-6), 37.9 (C-4), 51.0 (OCH_3), 70.5 (CH_2Ph), 78.0 (C-5), 119.2 (C-2), 127.3 (Ar-C), 127.6 (2 × Ar-C), 128.2 (2 × Ar-C), 139.0 (Ar-C_{ipso}), 152.8 (C-3), 166.7 (C-1); *m/z* (ESI) 271 (MNa^+, 100%), 177 (14) and 125 (11); Found (ESI): 271.1313 (MNa^+), ($C_{15}H_{20}O_3Na$ requires 271.1305). *(E)-methyl ester* **194**: $[\alpha]_D^{20}$ +20.1 (*c* 2.59, $CHCl_3$); ν_{max}(neat)/cm^{-1} 2973, 2874, 1720 (C=O), 1656 (C=C), 1454, 1435, 1250, 1093, 734, 697; δ_H(400 MHz) 1.08 (3H, d, *J* 7.0, 4-CH_3), 1.14 (3H, d, *J* 6.0, 6-H_3), 2.54 (1H, m, 4-H), 3.50 (1H, dq, *J* 6.0, 5.5, 5-H), 3.73 (3H, s, OCH_3), 4.45 (1H, d, *J* 11.5, C*H*HPh), 4.58 (1H, d, *J* 11.5, CH*H*Ph), 5.84 (1H, dd, *J* 15.5, 1.5, 2-H), 6.99 (1H, dd, *J* 15.5, 7.5, 3-H), 7.24-7.34 (5H, m, 5 × Ar-H); δ_C(100 MHz) 14.6 (4-CH_3), 16.5 (C-6), 41.8 (C-4), 51.4 (OCH_3), 70.7 (CH_2Ph), 77.4 (C-5), 120.9 (C-2), 127.4 (Ar-C), 127.5 (2 × Ar-C), 128.3 (2 × Ar-C), 138.6 (Ar-C_{ipso}), 151.4 (C-3), 167.0 (C-1); *m/z* (ESI) 271 (MNa^+, 100%), 245 (14), 217 (9), 177 (22) and 125 (16); Found (ESI): 271.1313 (MNa^+), ($C_{15}H_{20}O_3Na$ requires 271.1305).

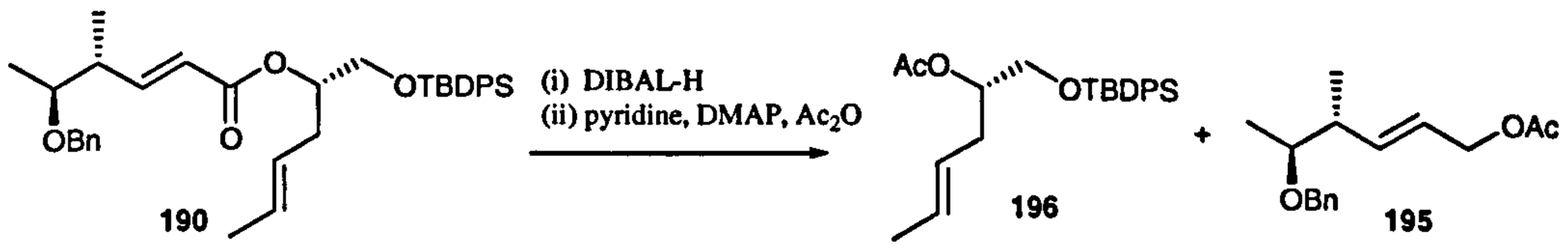
(1′*S*,3′*E*)-1′-*tert*-Butyldiphenylsilanoxymethylhex-3′-en-1′-yl (2*E*,4*R*,5*S*)-5-(benzyloxy)-4-methylhex-2-enoate 190



To a solution of acid **189** (0.95 g, 4.05 mmol) and alcohol **187** (1.05 g, 3.04 mmol) in DCM (44 mL) was added DCC (0.84 g, 4.05 mmol) in dry DCM (3 mL) and 4-DMAP (0.04 g, 0.33 mmol). The solution was stirred overnight and then hexane (50 mL) was added and the solution was filtered to remove the urea by-product. Solvents were removed *in vacuo* to give a yellow oil, which was purified by flash column chromatography (SiO_2, 0-5% ethyl acetate/hexane) to afford *ester* **190** (1.11 g, 66%) as a colourless oil. $[\alpha]_D^{22}$ +2.0 (*c* 2.03, $CHCl_3$); ν_{max}(neat)/cm^{-1} 2963, 2931, 2857,

1717 (C=O), 1652 (C=C), 1589 (C=C), 1428, 1261, 1112, 968, 823, 739, 700; δ_H(400 MHz) 1.03 (9H, s, $SiC(CH_3)_3$), 1.09 (3H, d, *J* 7.0, 4-CH_3), 1.13 (3H, d, *J* 6.5, 6-H_3), 1.61 (3H, dd, *J* 6.5, 1.5, 5′-H_3), 2.33 (1H, m, 2′-*H*H), 2.42 (1H, m, 2′-H*H*), 2.57 (1H, m, 4-H), 3.51 (1H, dq, *J* 6.5, 5.0, 5-H), 3.72 (2H, dd, *J* 5.0, 1.0, 1′-CH_2), 4.47 (1H, d, *J* 12.0, OC*H*HPh), 4.58 (1H, d, *J* 12.0, OCH*H*Ph), 5.03 (1H, app. tt, *J* 6.5, 5.0, 1′-H), 5.34 (1H, dtq, *J* 15.0, 7.0, 1.5, 3′-H), 5.50 (1H, dqt, *J* 15.0, 6.5, 1.0, 4′-H), 5.83 (1H, dd, *J* 16.0, 1.5, 2-H), 6.98 (1H, dd, *J* 16.0, 7.5, 3-H), 7.23-7.44 (11H, m, 11 × Ar-H), 7.63-7.68 (4H, m, 4 × Ar-H); δ_C(100 MHz) 14.5 (4-CH_3), 16.4 (C-6), 18.0 (C-6′), 19.2 ($SiC(CH_3)_3$), 26.7 ($SiC(CH_3)_3$), 33.8 (C-2′), 41.7 (C-4), 64.3 (1′-CH_2), 70.7 ($PhCH_2O$), 73.8 (C-1′), 77.4 (C-5), 121.5 (C-2), 125.7 (C-3′ or C-4′), 127.5 (Ar-C), 127.6 (2 × Ar-C), 127.6 (2 × Ar-C), 127.6 (2 × Ar-C), 128.3 (2 × Ar-C), 128.4 (C-3′ or C-4′), 129.6 (2 × Ar-C), 133.4 (Ar-C_{ipso}), 133.5 (Ar-C_{ipso}), 135.6 (2 × Ar-C), 135.6 (2 × Ar-C), 138.7 (Ar-C_{ipso}), 151.1 (C-3), 166.0 (C-1); *m/z* (ESI) 593 (MNa^+, 100%), 588 (26), 571 (7), 493 (14) and 198 (10); Found (ESI): 593.3080 (MNa^+), ($C_{36}H_{46}O_4SiNa$ requires 593.3058).

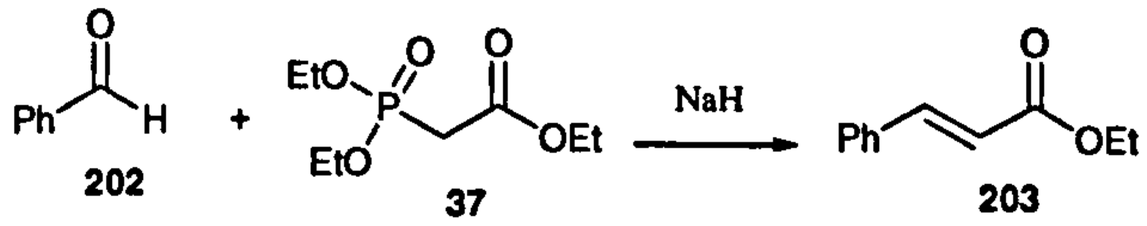
(2*S*,4*E*)-2-Acetoxy-1-*tert*-butyldiphenylsilanoxyhex-4-ene 196 and (2*E*,4*R*,5*S*)-1-Acetoxy-5-(benzyloxy)-4-methylhex-2-ene 195



Ester **190** (0.05 g, 0.09 mmol) was dissolved in dry DCM (6 mL) and cooled to -78 °C. Diisobutylaluminium hydride (1.0 M in hexanes, 0.18 mL, 0.18 mmol) was added dropwise and the solution was stirred for 45 minutes. Pyridine (0.02 mL, 0.27 mmol), DMAP (0.03 g, 0.27 mmol) in of dry DCM (1 mL) and acetic anhydride (0.05 mL, 0.54 mmol) were added sequentially dropwise. The mixture was stirred for 14.5 hours at -78 °C and was then allowed to warm gradually to 0 °C. Saturated aqueous ammonium chloride solution (5 mL) and saturated aqueous potassium sodium L-tartrate tetrahydrate solution (5 mL) were added and the resulting mixture was allowed to warm to room temperature and stirred for 2 hours. The layers were then separated and the aqueous phase was extracted with DCM (3 × 50 mL). The combined organic extracts were washed with saturated aqueous sodium sulfate solution (10 mL) and saturated aqueous sodium hydrogen carbonate solution (10 mL),

dried over magnesium sulfate, filtered and concentrated *in vacuo* to give a yellow oil. This was purified by flash column chromatography (SiO_2, 2.5-5% ethyl acetate/hexane) to afford *silyl ether* **196** (0.0287 g, 83%) and *acetate* **195** (0.0147 g, 64%) both as colourless oils. *Silyl ether* **196**: $[\alpha]_D^{23}$ -6.9 (*c* 1.44, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3072, 2932, 2858, 1739 (C=O), 1428, 1373, 1237, 1112, 968, 823, 740, 701; δ_H(400 MHz) 1.08 (9H, s, $SiC(CH_3)_3$), 1.66 (3H, dq, *J* 6.5, 1.5, 6-H_3), 2.05 (3H, s, $C(O)CH_3$), 2.30 (1H, app. dtt, *J* 14.0, 7.0, 1.0, 3-*H*H), 2.40 (1H, app. dtt, *J* 14.0, 7.0, 1.0, 3-H*H*), 3.72 (2H, dd, *J* 5.0, 1.0, 1-H_2), 5.00 (1H, app. tt, *J* 6.5, 5.0, 2-H), 5.35 (1 H, dtq, *J* 15.0, 7.0, 1.5, 4-H), 5.53 (1H, dqt, *J* 15.0, 6.5, 1.0, 5-H), 7.39-7.49 (6H, m, 6 × Ar-H), 7.67-7.72 (4H, m, 4 × Ar-H); δ_C(100 MHz) 18.0 (C-6), 19.2 ($SiC(CH_3)_3$), 21.2 ($C(O)CH_3$), 26.7 ($SiC(CH_3)_3$), 33.8 (C-3), 64.3 (C-1), 74.1 (C-2), 125.7 (C-4 or C-5), 127.6 (2 × Ar-C), 127.7 (2 × Ar-C), 128.4 (C-4 or C-5), 129.6 (Ar-C), 129.7 (Ar-C), 133.4 (Ar-C_{ipso}), 133.5 (Ar-C_{ipso}), 135.5 (2 × Ar-C), 135.6 (2 × Ar-C), 170.5 (CO); *m/z* (ESI) 419 (MNa^+, 100%), 325 (8), 285 (41) and 139 (11); Found (ESI): 419.2025 (MNa^+), ($C_{24}H_{32}O_3SiNa$ requires 419.2013). *Acetate* **195**: $[\alpha]_D^{23}$ +16.2 (*c* 0.74, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3032, 2969, 2932, 2872, 1737 (C=O), 1454, 1375, 1226, 1094, 1026, 972, 736, 698; δ_H(400 MHz) 1.03 (3H, d, *J* 7.0, 4-CH_3), 1.12 (3H, d, *J* 6.0, 6-H_3), 2.05 (3H, s, $C(O)CH_3$), 2.41 (1H, m, 4-H), 3.42 (1H, app. quint., *J* 6.0, 5-H), 4.45 (1H, d, *J* 12.0, C*H*HPh), 4.53 (2H, d, *J* 6.5, 1-H_2), 4.57 (1H, d, *J* 12.0, CH*H*Ph), 5.58 (1H, dtd, *J* 15.5, 6.5, 1.0, 2-H), 5.76 (1H, ddt, *J* 15.5, 7.5, 1.0, 3-H), 7.25-7.40 (5H, m, 5 × Ar-H); δ_C(100 MHz) 15.1 (4-CH_3), 16.3 (C-6), 21.0 ($C(O)CH_3$), 41.5 (C-4), 65.5 (C-1), 70.7 (CH_2Ph), 78.1 (C-5), 123.9 (C-2), 127.4 (Ar-C), 127.5 (2 × Ar-C), 128.3 (2 × Ar-C), 138.3 (C-3), 138.9 (Ar-C_{ipso}), 170.9 (CO); *m/z* (ESI) 285 (MNa^+, 100%); Found (ESI): 285.1460 (MNa^+), ($C_{16}H_{22}O_3Na$ requires 285.1461).

Ethyl (*E*)-3-phenylpropenoate 203[193]

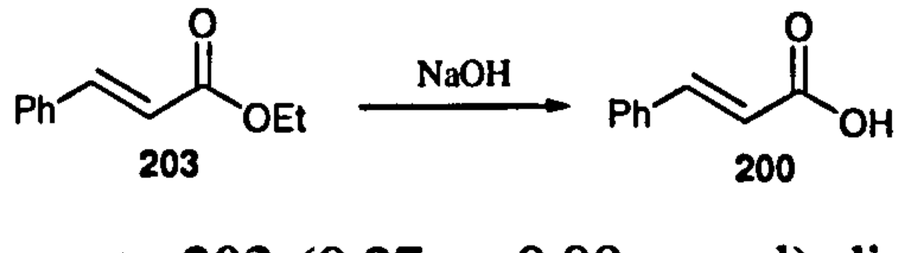


Sodium hydride (60% dispersion oil prewashed in hexane, 0.12 g, 3.00 mmol) was stirred in dry THF (10 mL) under an atmosphere of nitrogen. Triethyl phosphonoacetate **37** (0.58 g, 2.60 mmol) in dry THF (3 mL) was added dropwise and

stirred for 25 minutes. Then benzaldehyde **202** (0.25 g, 2.36 mmol) in THF (3 mL) was added dropwise over a period of approximately 30 minutes (in order to prevent a gum like precipitate which had previously been observed). The resulting solution was stirred for 20 hours at room temperature. The reaction mixture was then quenched with water (100 mL) and was extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo*. Purification by flash column chromatography (SiO_2, 20% ethyl acetate/petroleum ether 40-60 °C) afforded unsaturated ester **203** (0.27 g, 67%) as a yellow oil. δ_H(400 MHz) 1.33 (3H, t, *J* 7.0, CH_3), 4.34 (2H, q, *J* 7.0, OCH_2), 6.45 (1H, d, *J* 16.0, 2-H), 7.34-7.45 (3H, m, 3 × Ar-H), 7.50-7.57 (2H, m, 2 × Ar-H), 7.80 (1H, d, *J* 16.0, 3-H); δ_C(100 MHz) 14.4 (CH_3), 60.5 (OCH_2), 118.4 (C-2), 128.1 (2 × Ar-C), 128.9 (2 × Ar-C), 130.3 (Ar-C), 134.5 (Ar-C_{ipso}), 144.6 (C-3), 167.0 (C-1).

(*E*)-3-Phenylpropanoic Acid (Cinnamic Acid) 200[194]

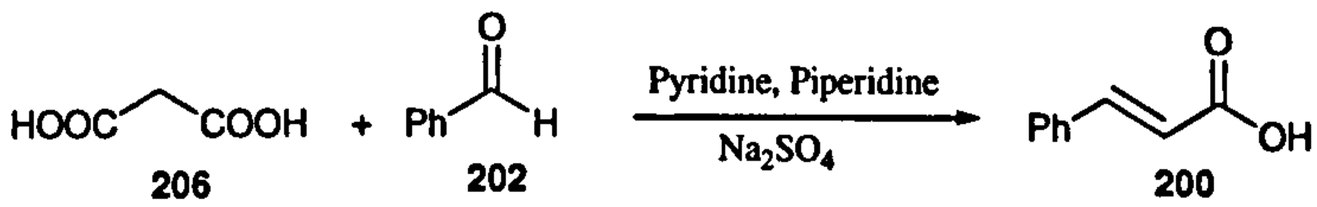
(a) *via* hydrolysis of ethyl ester 203



203 **200**

Ethyl (*E*)-3-phenylpropenoate **203** (0.27 g, 0.99 mmol) dissolved in ethanol (1 mL) was added dropwise to sodium hydroxide (2.0 M, 10 mL) and heated to reflux for 24 hours. The mixture was quenched with water (40 mL) and acidified to pH 2 with hydrochloric acid (2.0 M, 30 mL). The solution was then extracted with ethyl acetate (3 × 100 mL), dried over sodium sulfate, filtered and concentrated *in vacuo* to yield cinnamic acid **200** (0.17 g, 100%) as a white solid. m.p. 132-134 °C, lit.[120] 132-135 °C; δ_H(400 MHz) 6.46 (1H, d, *J* 16.0, 2-H), 7.34-7.45 (3H, m, 3 × Ar-H), 7.50-7.57 (2H, m, 2 × Ar-H), 7.80 (1H, d, *J* 16.0, 3-H); δ_C(100 MHz) 117.3 (C-2), 128.4 (2 × Ar-C), 129.0 (2 × Ar-C), 130.8 (Ar-C), 134.1 (Ar-C_{ipso}), 147.1 (C-3), 171.8 (C-1); *m/z* (EI) 149 (MH^+, 99%), 131 (74), 107 (100), 91 (15) and 84 (9).

(b) *via* Knoevenagel Reaction



206 **202** **200**

Malonic acid **206** (0.25 g, 2.40 mmol), benzaldehyde **202** (0.25 g, 2.36 mmol), pyridine (0.25 mL), piperidine (15 μL) and sodium sulfate (0.05 g, 1.25 mmol) were

refluxed for 4 hours. The solution was acidified with concentrated hydrochloric acid and the white precipitate which formed was dissolved by adding diethyl ether (20 mL). The organic phase was extracted with sodium hydroxide (2.0 M, 3 × 20 mL) and the combined aqueous extracts were acidified with concentrated hydrochloric acid and then filtered to furnish cinnamic acid **200** (0.27 g, 87%) as shiny white crystals. These were used without further purification. Spectral data as before.

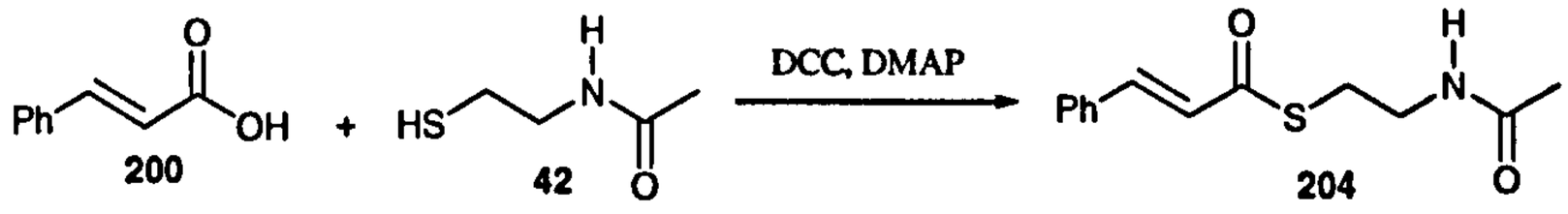
[3-^{13}C]-(*E*)-Cinnamic Acid 6[120]

The above Knoevenagel reaction was repeated using [1-^{13}C]-benzaldehyde **205** (0.50 g, 4.72 mmol), to give acid **6** (0.70 g, 100%). δ_H(400 MHz) 6.47 (1H, dd, *J* 16.0, 1.0, 2-H), 7.39-7.44 (3H, m, 3 × Ar-H), 7.54-7.59 (2H, m, 2 × Ar-H), 7.81 (1H, dd, *J* 156.5, 16.0, 3-H); δ_C(100 MHz) signal assigned to C-3 (147.1 ppm) showed an enhancement of >95% (based on ^{1}H NMR and MS data); *m/z* (CI) 150 (MH^+, 65%), 132 (32), 106 (8), 93 (11), 79 (6), 75 (100), 65 (20) and 59 (19).

[2,3-$^{13}C_2$]-(2*E*)-Cinnamic Acid 209

The above Knoevenagel reaction was repeated using [1-^{13}C]-benzaldehyde **205** (0.25 g, 2.36 mmol) and [2-^{13}C]-malonic acid **207** (0.25 g, 2.40 mmol) to give *acid* **209** (0.20 g, 57%). δ_H(400 MHz) 6.47 (1H, ddd, *J* 163.0, 16.0, 1.0, 2-H), 7.39-7.44 (3H, m, 3 × Ar-H), 7.54-7.59 (2H, m, 2 × Ar-H), 7.81 (1H, ddd, *J* 156.5, 16.0, 3.0, 3-H); δ_C(100 MHz) signals assigned to C-2 (117.3 ppm) and C-3 (147.1 ppm) appear as doublets (*J* 70.5) with an enhancement of >90% (based on ^{1}H NMR and MS data); *m/z* (EI) 150 (M^+, 91%), 149 (100), 133 (46), 105 (68), 92 (43), 84 (30), 78 (50) and 63 (25).

Cinnamic Acid *N*-Acetylcysteamine Thiol Ester 204[195]



Freshly prepared *N*-acetylcysteamine **42** (0.09 g, 0.78 mmol) in dry DCM (5 mL) was stirred at 0 °C under an atmosphere of nitrogen. DCC (0.12 g, 0.60 mmol) in dry DCM (1 mL) was added followed by DMAP (0.003 g, 0.02 mmol) in dry DCM (1 mL). This was stirred for 10 minutes before addition of cinnamic acid **200** (0.07 g, 0.53 mmol). The solution was left at 0 °C for 2 hours and was allowed to warm to

room temperature overnight. The reaction was subsequently quenched with saturated aqueous ammonium chloride solution (100 mL) and was extracted with DCM (3 × 100 mL). The combined organic extracts were dried over magnesium sulfate, and copper sulfate impregnated silica (prepared by stirring flash silica in a saturated solution of copper sulfate for 10 minutes and then drying *in vacuo* to a free flowing powder)[170] was added to remove any unreacted *N*-acetylcysteamine **42**. The solution was then filtered and concentrated *in vacuo* to yield a white solid. Ethyl acetate (3 mL) was added to dissolve the product and the insoluble urea by-product was then filtered off. The filtrate was then concentrated *in vacuo* give thiol ester **204** (0.09 g, 68%) as an off white solid. m.p. 91-94 °C (from ethyl acetate/petroleum ether 40-60 °C), lit.[195] 97-98 °C; δ_H(400 MHz) 1.99 (3H, s, CH_3), 3.17 (2H, t, *J* 6.5, CH_2S), 3.51 (2H, app. q, *J* 6.5, CH_2N), 6.26 (1H, br s, NH), 6.73 (1H, d, *J* 16.0, 2-H), 7.36-7.43 (3H, m, 3 × Ar-H), 7.51-7.56 (2H, m, 2 × Ar-H), 7.62 (1H, d, *J* 16.0, 3-H); δ_C(100 MHz) 23.3 ($COCH_3$), 28.6 (CH_2S), 39.8 (CH_2N), 124.7 (C-2), 128.5 (2 × Ar-C), 129.1 (2 × Ar-C), 130.9 (Ar-C), 133.9 (Ar-C_{ipso}), 141.3 (C-3), 170.5 (CON), 190.2 (C-1); *m/z* (EI) 249 (MH^+, 28%), 221 (63), 190 (75), 162 (14), 131 (100), 103 (40), 77 (33) and 56 (10).

[3-^{13}C]-Cinnamic Acid *N*-Acetylcysteamine Thiol Ester 6[120]

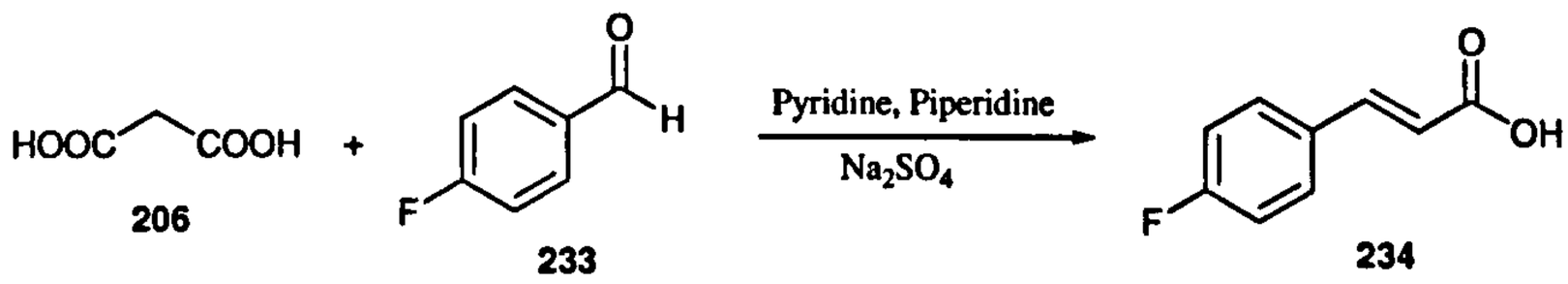
The above was repeated using [3-^{13}C]-cinnamic acid **208** (0.20 g, 1.34 mmol) to furnish the thiol ester **6** (0.23 g, 60%) as an off white solid. δ_H(400 MHz) 1.99 (3H, s, CH_3), 3.18 (2H, t, *J* 6.5, CH_2S), 3.52 (2H, app. q, *J* 6.5, CH_2N), 5.99 (1H, br s, NH), 6.74 (1H, d, *J* 16.0, 2-H), 7.27-7.42 (3H, m, 3 × Ar-H), 7.54-7.57 (2H, m, 2 × Ar-H), 7.64 (1H, dd, *J* 156.0, 16.0, 3-H); δ_C(100 MHz) signal assigned to C-3 (141.3 ppm) showed an enhancement of >95% (based on ^{1}H NMR and MS data); *m/z* (EI) 250 (M^+, 15%), 222 (35), 191 (46), 132 (100), 104 (43), 78 (24) and 77 (25).

[2,3-$^{13}C_2$]-Cinnamic Acid *N*-Acetylcysteamine Thiol Ester 7

The above was repeated using [2,3-$^{13}C_2$]-cinnamic acid **209** (0.20 g, 1.33 mmol) to yield the crude product as a yellow solid. The product was purified by flash column chromatography (SiO_2, 50%-100% ethyl acetate/petroleum ether 40-60 °C) furnishing the *thiol ester* **7** (0.25 g, 67%) and a slight impurity believed to be *N,S*-diacetylcysteamine **240** as fine white needles. However, a small analytical sample was

later purified by flash column chromatography (SiO_2, 2.5% methanol/ethyl acetate, followed by SiO_2, 50%-100% ethyl acetate/petroleum ether 40-60 °C). δ_H(400 MHz) 1.99 (3H, s, CH_3), 3.17 (2H, t, *J* 6.5, CH_2S), 3.51 (2H, app. q, *J* 6.5, CH_2N), 6.10 (1H, br s, NH), 6.73 (1H, ddd, *J* 161.0, 16.0, 1.5, 2-H), 7.35-7.43 (3H, m, 3 × Ar-H), 7.53-7.58 (2H, m, 2 × Ar-H), 7.65 (1H, ddd, *J* 155.5, 16.0, 2.5, 3-H); δ_C(100 MHz) signals assigned to C-2 (124.7 ppm) and C-3 (141.3 ppm) appear as doublets (*J* 70.5) with an enhancement of >90% (based on ^{1}H NMR and MS data); *m/z* (EI) 251 (M^+, 21%), 223 (64), 192 (84), 164 (19), 143 (27), 133 (100), 105 (58) and 78 (26).

(*E*)-*p*-Fluorocinnamic Acid 234[196]



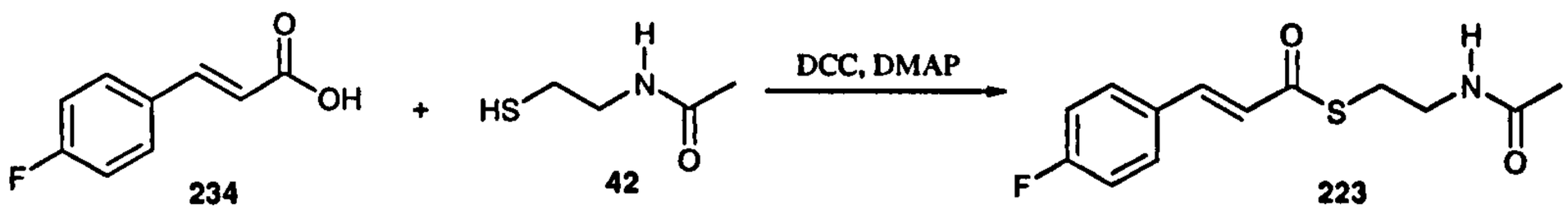
Malonic acid **206** (0.26 g, 2.50 mmol), *p*-fluorobenzaldehyde **233** (0.35 g, 2.82 mmol), pyridine (0.25 mL), piperidine (15 µL) and sodium sulfate (0.05 g, 1.25 mmol) were refluxed for 4 hours. The solution was acidified with concentrated hydrochloric acid and the white precipitate which formed was dissolved in diethyl ether (20 mL). The organic phase was extracted with sodium hydroxide (2.0 M, 3 × 20 mL) and the combined aqueous extracts were acidified with concentrated hydrochloric acid and then filtered to furnish (2*E*)-*p*-fluorocinnamic acid **234** (0.42 g, 100%) as an off white powdery solid. This was used without further purification. m.p. 209-211 °C (from MeOH), lit.[196] 205-207 °C; δ_H(400 MHz) 6.38 (1H, d, *J* 16.0, 2-H), 7.10 (2H, t, *J* 8.5, 2 × CHCF), 7.52-7.57 (2H, m, 2 × Ar-H), 7.75 (1H, d, *J* 16.0, 3-H); δ_C(100 MHz) 116.3 (d, *J* 22.5, 2 × Ar-C), 117.0 (d, *J* 2.5, C-2), 130.1 (d, *J* 3.0, Ar-C_{ipso}), 130.4 (d, *J* 8.5, 2 × Ar-C), 145.8 (C-3), 164.2 (d, *J* 253.0, C-F), 171.3 (C-1); *m/z* (CI) 167 (MH^+, 100%), 166 (66), 149 (80), 121 (10), 109 (6) and 79 (11).

[2-^{13}C]-(*E*)-*p*-Fluorocinnamic Acid 235

The above reaction was repeated using [2-^{13}C]-malonic acid **207** (0.25 g, 2.40 mmol) to give *acid* **235** (0.33 g, 83%). δ_H(400 MHz) 6.38 (1H, dd, *J* 162.5, 16.0, 2-H), 7.06 (2H, t, *J* 8.5, 2 × CHCF), 7.52-7.58 (2H, m, 2 × Ar-H), 7.75 (1H, dd, *J* 16.0, 3.0, 3-H); δ_C(100 MHz) signal assigned to C-2 (117.0 ppm, d, *J* 2.5) showed an

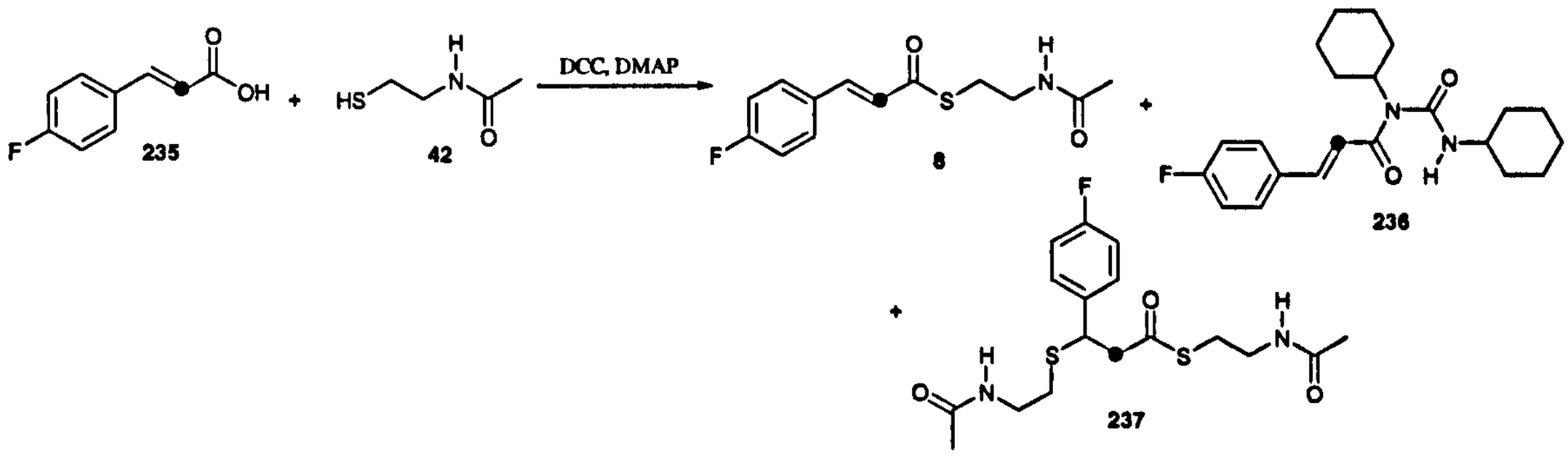
enhancement of >90% (based on ^{1}H NMR and MS data); *m/z* (EI) 167 (M^+, 100%), 166 (48), 150 (44), 122 (39), 109 (26), 102 (38), 86 (46) and 84 (72).

(*E*)-*p*-Fluorocinnamic Acid *N*-Acetylcysteamine Thiol Ester 223



Freshly prepared *N*-acetylcysteamine **42** (0.09 g, 0.78 mmol) in dry DCM (5 mL) was stirred at 0 °C under an atmosphere of nitrogen. DCC (0.12 g, 0.60 mmol) in dry DCM (1 mL) was added followed by DMAP (0.003 g, 0.02 mmol) in dry DCM (1 mL). This was stirred for 10 minutes before addition of *p*-fluorocinnamic acid **234** (0.07 g, 0.53 mmol). The solution was left at 0 °C for 2 hours and was allowed to warm to room temperature overnight. The reaction was subsequently quenched with saturated aqueous ammonium chloride solution (100 mL) and extracted with DCM (3 × 100 mL). The combined organic extracts were dried over magnesium sulfate, and copper sulfate impregnated silica (prepared by stirring flash silica in a saturated solution of copper sulfate for 10 minutes and then drying *in vacuo* to a free flowing powder)[170] was added to remove any unreacted *N*-acetylcysteamine **42.** The solution was then filtered and concentrated *in vacuo* to yield a white solid. Ethyl acetate was added (3 mL) to dissolve the product and the insoluble urea by-product was then filtered off. The filtrate was removed *in vacuo* and the resulting solid was purified by flash column chromatograpy (SiO_2, 2.5% methanol/ethyl acetate, followed by SiO_2, 50%-100% ethyl acetate/petroleum ether 40-60 °C) to furnish the *thiol ester* **223** (0.09 g, 68%) as an off white solid. Found: C, 58.43; H, 5.02; F, 6.91; N, 4.95; S, 11.93; $C_{13}H_{14}FNO_2S$ requires C, 58.41; H, 5.28; F, 7.11; N, 5.24; S, 12.00; m.p. 152-154 °C (from MeOH); ν_{max}(neat)/cm^{-1} 3287 (CONH), 3073 (CONH), 2923, 1665, 1651, 1614, 1591, 1544, 1506, 1226, 1160, 1042, 978, 815, 737; δ_H(400 MHz) 1.98 (3H, s, CH_3), 3.17 (2H, t, *J* 6.5, CH_2S), 3.51 (2H, app. q, *J* 6.5, CH_2N), 5.89 (1H, br s, NH), 6.64 (1H, d, *J* 15.5, 2-H), 7.10 (2H, t, *J* 8.5, 2 × C*H*CF), 7.52-7.57 (2H, m, 2 × Ar-H), 7.59 (1H, d, *J* 15.5, 3-H); δ_C(100 MHz) 23.3 (CH_3), 28.6 (CH_2S), 39.8 (CH_2N), 116.4 (d, *J* 22.5, 2 × Ar-C), 124.4 (d, *J* 2.5, C-2), 130.2 (d, *J* 3.0, Ar-C_{ipso}), 130.6 (d, *J* 9.0, 2 × Ar-C), 140.0 (C-3), 164.2 (C-F, d, *J* 253.0), 170.4 (CON), 190.1 (C-1); *m/z* (EI) 267 (M^+, 30%), 239 (53), 208 (76), 150 (46), 149 (100), 121 (60), 101 (50) and 86 (42).

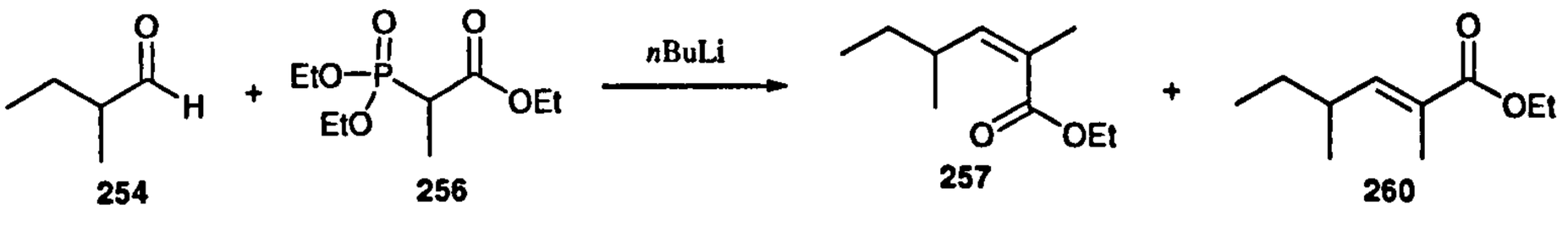
[2-^{13}C]-(*E*)-*p*-Fluorocinnamic Acid *N*-Acetylcysteamine Thiol Ester 8



The above was repeated using [2-^{13}C]-*p*-fluorocinnamic acid **235** (0.30 g, 1.34 mmol) to furnish the *thiol ester* **8** (0.12 g, 23%) as an off white solid and the *by-products* **236** (0.07g) and **237** (0.12g) as a white solid and an orange oil respectively. *Thiol ester* **8**: δ_H(400 MHz) 1.98 (3H, s, CH_3), 3.17 (2H, t, *J* 6.5, CH_2S), 3.51 (2H, app. q, *J* 6.5, CH_2N), 6.01 (1H, br s, NH), 6.66 (1H, dd, *J* 161.0, 15.5, 2-H), 7.10 (2H, app. t, *J* 8.5, 2 × C*H*CF), 7.52-7.57 (2H, m, 2 × Ar-H), 7.59 (1H, dd, *J* 15.5, 2.5, 3-H); δ_C(100 MHz) signal assigned to C-2 (124.4 ppm, d, *J* 2.5) showed an enhancement of >90% (based on ^{1}H NMR and MS data); *m/z* (EI) 268 (M^+, 27%), 240 (50), 209 (75), 150 (100), 122 (70), 102 (58), 86 (33) and 76 (27). *N-Acyl urea* **236**: m.p. 169-170 °C (from ethyl acetate/petroleum ether 40-60 °C), ν_{max}(neat)/cm^{-1} 3259 (CONH), 3045 (CONH), 2932, 2854, 1703 (C=O), 1633 (C=O), 1508, 1374, 1227, 1162, 994, 985, 831, 810; δ_H(400 MHz) 0.80-2.05 (20H, m, 10 × CH_2), 3.76 and 4.09 (each 1H, each m, each NCH), 6.66 (1H, dd, *J* 158.5, 15.5, 2-H), 6.92 (1H, br s NH), 7.07 (2H, app. t, *J* 8.5, 2 × C*H*CF), 7.44-7.50 (2H, m, 2 × Ar-H), 7.64 (1H, dd, *J* 15.5, 3.5, 3-H); δ_C(100 MHz) signal assigned to C-2 (119.2 ppm) showed an enhancement of >90% (based on ^{1}H NMR and MS data); *m/z* (ESI) 396 (MNa^+, 100), 374 (28), 265 (29), 256 (14), 185 (7) and 132 (11); Found (ESI) 396.2140 (MNa^+), ($C_{21}{}^{13}CH_{29}O_2N_2FNa$ requires 396.2139). *Addition compound* **237**: ν_{max}(neat)/cm^{-1} 3285 (CONH), 3079 (CONH), 2931, 1646 (C=O), 1548 (C=O), 1508 (C=O), 1432, 1374, 1290, 1222, 1039, 975, 725; δ_H(400 MHz) 1.94 and 1.97 (each 3H, each s, each CH_3), 2.47 (2H, t, *J* 7.0, CH_2S), 2.83 (2H, t, *J* 6.5, CH_2S), 3.00 (2H, m, 2-H_2), 3.30-3.40 (2H, m, CH_2N), 3.55 (2H, app. q, *J* 6.5, CH_2N), 4.35 (1H, dt, *J* 7.5, 5.0, 3-H), 6.26 and 6.33 (each 1H, each br s, each NH), 7.02 (2H, app. t, *J* 8.5, 2 × C*H*CF), 7.32 (2H, m, 2 × Ar-H); δ_C(100 MHz) signal assigned to C-2 (50.2 ppm) showed an enhancement of >90% (based on ^{1}H NMR and MS data); *m/z* (EI) 388 (M^+, 56), 270 (46), 150 (100), 123

(41), 118 (52), 102 (34), 86 (52) and 60 (38); Found (EI): 388.1254 (M^+), ($C_{16}{}^{13}CH_{24}FN_2O_3S_2$ requires 388.1246).

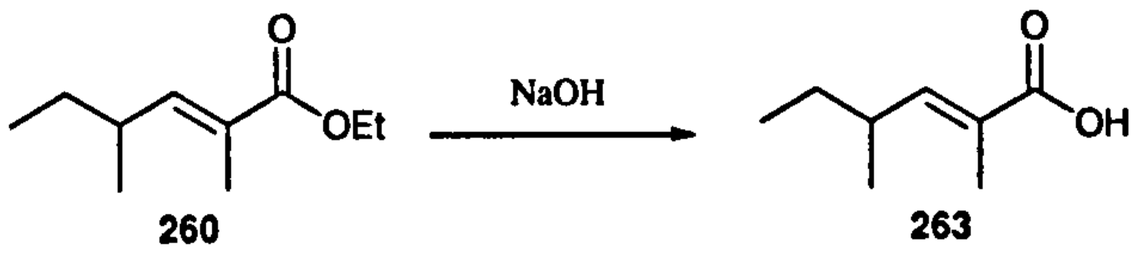
Ethyl (Z)-2,4-Dimethylhex-2-enoate 257[158] and Ethyl (*E*)-2,4-Dimethylhex-2-enoate 260[158]



254 256 257 260

Triethyl 2-phosphonopropionate **256** (2.04 mL, 9.53 mmol) in dry THF (50 mL) under an atmosphere of nitrogen was cooled to 0 °C. *n*-Butyllithium (2.5 M in hexanes, 3.81 mL, 9.53 mmol) was added slowly and the mixture was stirred for 1 hour. 2-Methylbutanal **254** (1.00 mL, 9.33 mmol) was then added dropwise and the resulting solution was stirred at room temperature for 17 hours. The reaction was quenched with water (40 mL), extracted with ethyl acetate (3 × 100 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield a colourless oil. Purification by flash column chromatography (SiO_2, 2% ethyl acetate/petroleum ether 40-60 °C) furnished (*Z*)-ethyl ester **257** (1.04 g, 65%) and (*E*)-ethyl ester **260** (0.43 g, 27%), both as colourless oils. (*Z*)-Ethyl ester **257**: δ_H(400 MHz) 0.86 (3H, t, *J* 7.5, 6-H_3), 0.98 (3H, d, *J* 6.5, 4-CH_3), 1.22-1.40 (2H, m, 5-H_2), 1.30 (3H, t, *J* 7.0, OCH_2CH_3), 1.90 (3H, d, *J* 1.5, 2-CH_3), 3.01 (1H, m, 4-H), 4.20 (2H, q, *J* 7.0, OCH_2CH_3), 5.65 (1H, dq, *J* 10.0, 1.5, 3-H); δ_C(100 MHz) 11.8 (C-6), 14.3 (OCH_2CH_3), 20.2 and 20.8 (2-CH_3 and 4-CH_3), 30.1 (C-5), 35.0 (C-4), 60.0 (OCH_2CH_3), 126.1 (C-2), 148.3 (C-3), 168.4 (C-1). (*E*)-Ethyl ester **260**: δ_H(400 MHz) 0.85 (3H, t, *J* 7.5, 6-H_3), 0.99 (3H, d, *J* 6.5, 4-CH_3), 1.25-1.45 (2H, m, 5-H_2), 1.29 (3H, t, *J* 7.0, OCH_2CH_3), 1.83 (3H, d, *J* 1.5, 2-CH_3), 2.39 (1H, m, 4-H), 4.19 (2H, q, *J* 7.0, OCH_2CH_3), 6.53 (1H, dq, *J* 10.0, 1.5, 3-H); δ_C(100 MHz) 11.9 (C-6), 12.5 (OCH_2CH_3), 14.3 (2-CH_3), 19.7 (4-CH_3), 29.7 (C-5), 34.9 (C-4), 60.4 (OCH_2CH_3), 126.6 (C-2), 147.8 (C-3), 168.5 (C-1).

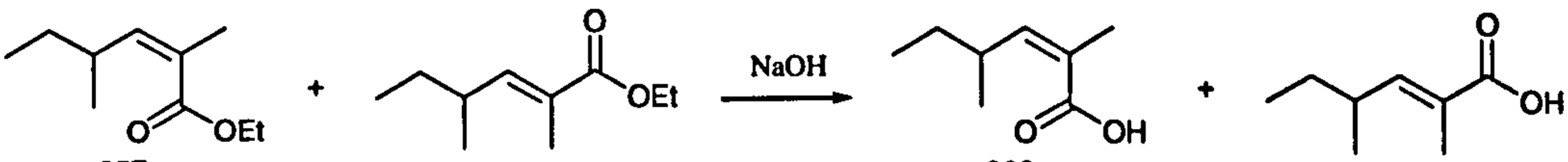
(*E*)-2,4-Dimethylhex-2-enoic Acid 263[197]



260 263

Unsaturated ester **260** (0.22 g, 1.29 mmol) in ethanol (1 mL) was added dropwise into sodium hydroxide (2.0 M, 15 mL). The solution was heated to reflux for 17 hours. The reaction was quenched with water (30 mL) and was acidified to pH 2 with hydrochloric acid (2.0 M). The solution was stirred for 10 minutes and was then saturated with sodium chloride. The mixture was extracted with ethyl acetate (3 × 50 mL) and the combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield acid **263** as a colourless oil (0.20 g, 100%). This was used without further purification. δ_H(400 MHz) 0.86 (3H, t, *J* 7.5, 6-H_3), 1.01 (3H, d, *J* 6.5, 4-CH_3), 1.25-1.50 (2H, m, 5-H_2), 1.85 (3H, d, *J* 1.5, 2-CH_3), 2.44 (1H, m, 4-H), 6.70 (1H, dq, *J* 10.5, 1.5, 3-H), 12.16 (1H, br s, COOH); δ_C(100 MHz) 11.9 (C-6), 12.2 (2-CH_3), 19.5 (4-CH_3), 29.6 (C-5), 35.2 (C-4), 125.9 (C-2), 150.8 (C-3), 174.3 (C-1).

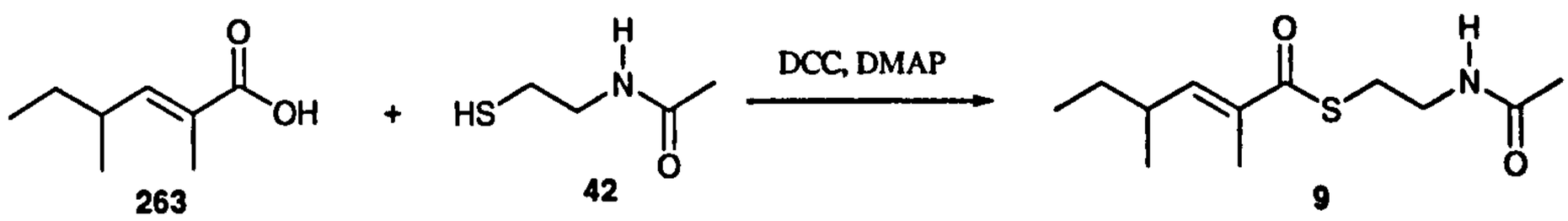
(*E*)-2,4-Dimethylhex-2-enoic Acid 263 and (*Z*)-2,4-Dimethylhex-2-enoic Acid 272[197,198]



257 260 263 272

The above procedure was repeated using a mixture of unsaturated esters **257** and **260** (1.17 g, 6.87 mmol) to furnish a mixture of unsaturated acids **263** and **272** (0.98 g, 100%) as a colourless oil. This was used without further purification. δ_H(400 MHz) 0.85 and 0.86 (each 3H, each t, *J* 7.5, 2 × 6-H_3), 0.97 and 1.01 (each 3H, each d, *J* 6.5, 2 × 4-CH_3), 1.15-1.50 (4H, m, 2 × 5-H_2), 1.85 and 1.92 (each 3H, each d, *J* 1.5, 2 × 2-CH_3), 2.44 and 3.16 (each 1H, each m, 2 × 4-H), 5.83 and 6.70 (each 1H, each dq, *J* 10.5, 1.5, 2 × 3-H), 11.75 (2H, br s, 2 × COOH); δ_C(100 MHz) 11.8 and 11.9 (2 × C-6), 12.2 (2-CH_3), 19.5 (4-CH_3), 20.2 and 20.7 (2-CH_3 and 4-CH_3), 29.6 and 29.7 (2 × C-5), 35.1 and 35.2 (2 × C-4), 125.0 and 125.8 (2 × C-2), 150.9 and 152.2 (2 × C-3), 174.1 and 174.2 (2 × C-1).

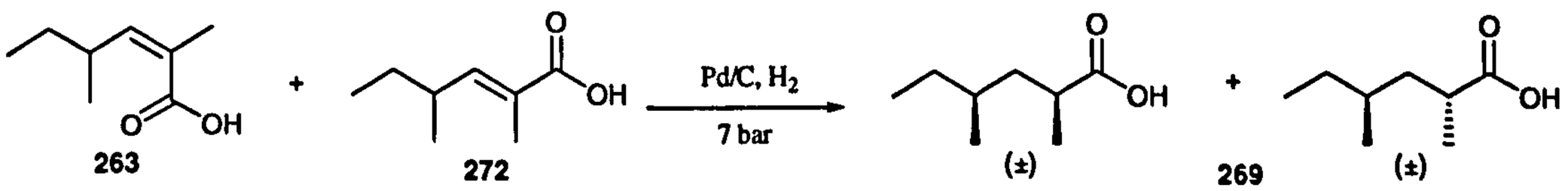
(*E*)-2,4-Dimethylhex-2-enoic Acid *N*-Acetylcysteamine Thiol Ester 9[199]



263 42 9

Acid **263** (0.18 g, 1.27 mmol) in dry DCM (40 mL) was stirred at 0 °C. DCC (0.34 g, 1.61 mmol) in dry DCM (3 mL) was added, followed by DMAP (0.008 g, 0.06 mmol) in dry DCM (3 mL). This was stirred for 15 minutes before addition of *N*-acetycysteamine **42** (0.28 g, 2.37 mmol) in dry DCM (4 mL). The solution was left at 0 °C for 2 hours and was then allowed to warm to room temperature overnight. The reaction was subsequently quenched with saturated aqueous ammonium chloride solution (10 mL) after 48 hours and was extracted with DCM (3 × 100 mL). The combined organic extracts were dried over magnesium sulfate, and copper sulfate impregnated silica (prepared by stirring flash silica in a saturated solution of copper sulfate for 10 minutes and then drying *in vacuo* to a free flowing powder)[170] was added to remove any unreacted *N*-acetylcysteamine **42**. The solution was then filtered and concentrated *in vacuo* to yield a white solid. DCM was added (3 mL) to dissolve the product and the insoluble urea by-product was then filtered off. The filtrate was concentrated *in vacuo* and purified by flash column chromatography (SiO_2, 50% ethyl acetate/petroleum ether 40-60 °C) to furnish the thiol ester **9** (0.15 g, 49%) as a colourless low melting solid. δ_H(400 MHz) 0.87 (3H, t, *J* 7.5, 6-H_3), 1.03 (3H, d, *J* 6.5, 4-CH_3), 1.27-1.52 (2H, m, 5-H_2), 1.89 (3H, d, *J* 1.5, 2-CH_3), 1.96 (3H, s, $COCH_3$), 2.44 (1H, m, 4-H), 3.06 (2H, t, *J* 6.5, CH_2S), 3.41-3.49 (2H, m, CH_2N), 6.05 (1H, br s, NH), 6.54 (1H, dq, *J* 10.0, 1.5, 3-H); δ_C(100 MHz) 11.8 (C-6), 12.6 (2-CH_3), 19.5 (4-CH_3), 23.1 ($COCH_3$), 28.5 (C-5), 29.6 (CH_2S), 35.1 (C-4), 39.8 (CH_2N), 134.6 (C-2), 147.4 (C-3), 170.2 (CON), 194.2 (C-1); *m/z* (ESI) 266 (MNa^+, 100%); Found (ESI): 266.1191 (MNa^+); ($C_{12}H_{21}NO_2SNa$ requires 266.1185).

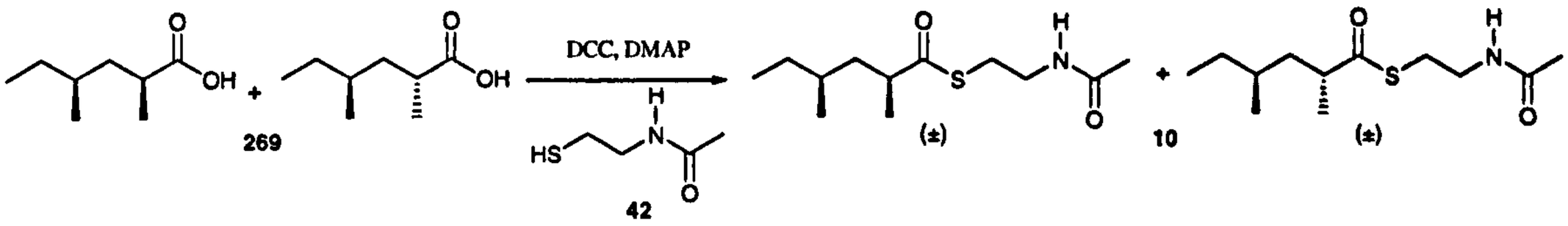
2,4-Dimethylhexanoic Acid 269[200]



A mixture of unsaturated acids **263** and **272** (0.98g, 6.89 mmol) was dissolved in methanol (10 mL) and palladium on carbon (≈ 20 mg) was added. The solution was stirred under an atmosphere of hydrogen (at a pressure of 7 bar) for 2 days. The catalyst was filtered off and washed with methanol. The solvent was then removed *in vacuo* to yield an approximately 1:1 mixture of the two diastereomeric pairs of acid **269** (0.97g, 100%) as a colourless oil. δ_H(400 MHz) 0.83-0.92 (12H, m, 2 × 4-CH_3

and 2 × 6-H_3), 1.10-1.21 (10H, m, 2 × 2-CH_3 and 2 × 5-H_2), 1.24-1.47 (4H, m, 2 × 3-*H*H and 2 × 4-H), 1.53 (1H, m, 3-H*H*), 1.74 (1H, ddd, *J* 13.5, 9.0, 5.5, 3-H*H*), 2.56 (2H, m, 2 × 2-H), 11.53 (2H, br s, 2 × COOH); δ_C(100 MHz) 11.1 and 11.2 (2 × C-6), 16.8 and 17.8 (2 × 2-CH_3), 18.8 and 19.0 (2 × 4-CH_3), 29.5 (2 × C-5), 32.3 (2 × C-4), 37.2 and 37.4 (2 × C-2), 40.4 and 40.9 (2 × C-3), 183.9 and 184.1 (2 × C-1).

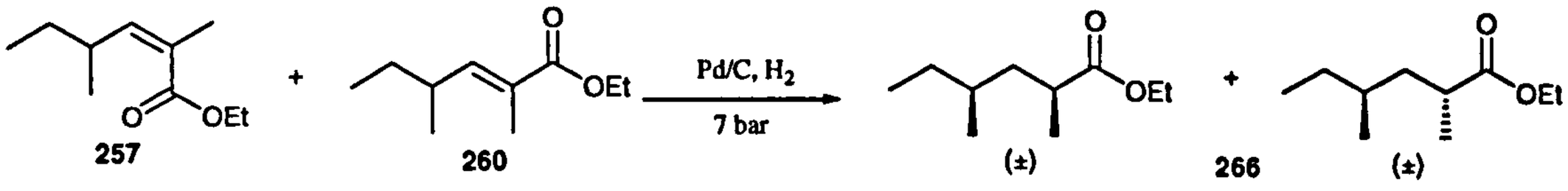
2,4-Dimethylhexanoic Acid *N*-Acetylcysteamine Thiol Ester 10



Acid **269** (0.17 g, 1.18 mmol) in dry DCM (40 mL) was stirred at 0 °C. DCC (0.34 g, 1.61 mmol) in dry DCM (3 mL) was added, followed by DMAP (0.008 g, 0.06 mmol) in dry DCM (3 mL). This was stirred for 15 minutes before addition of *N*-acetycysteamine **42** (0.28 g, 2.37 mmol) in dry DCM (4 mL). The solution was left at 0 °C for 2 hours and was then allowed to warm to room temperature overnight. The reaction was subsequently quenched with saturated aqueous ammonium chloride solution (30 mL) after 2 days and was extracted with DCM (3 × 100 mL). The combined organic extracts were dried over magnesium sulfate, and copper sulfate impregnated silica (prepared by stirring flash silica in a saturated solution of copper sulfate for 10 minutes and then drying *in vacuo* to a free flowing powder)[170] was added to remove any unreacted *N*-acetylcysteamine **42**. The solution was then filtered and concentrated *in vacuo* to yield a white solid. DCM was added (3 mL) to dissolve the product and the insoluble urea by-product was filtered off. The filtrate was then concentrated *in vacuo* and purified by flash column chromatography (SiO_2, 50% ethyl acetate/petroleum ether 40-60 °C) to furnish the two diastereomeric pairs of thiol ester **10** (0.22 g, 76%) as a colourless oil. ν_{max}(neat)/cm^{-1} 3288, 2963, 2930, 1688 (C=O), 1651 (C=O), 1549, 1454, 1374, 1289, 963, 753; δ_H(400 MHz) 0.78-0.87 (12H, m, 2 × 4-CH_3 and 2 × 6-H_3), 1.06-1.15 (10H, m, 2 × 2-CH_3 and 2 × 5-H_2), 1.21-1.39 (4H, m, 2 × 3-*H*H and 2 × 4-H), 1.47 (1H, m, 3-H*H*), 1.73 (1H, ddd, *J* 13.5, 9.0, 5.0, 3-H*H*), 1.93 (6H, s, 2 × $COCH_3$), 2.72 (2H, m, 2 × 2-H), 2.98 (4H, t, *J* 6.5, 2 × CH_2S), 3.39 (4H, q, *J* 6.5, 2 × CH_2N), 6.19 (2H, br s, 2 × NH); δ_C(100 MHz) 11.1 and 11.2 (2 × C-6), 17.6 and 18.7 (2 × 2-CH_3), 18.9 and 19.1 (2 × 4-CH_3), 23.2 ($COCH_3$), 28.1 (2 × C-5), 29.4 (2 × CH_2S), 32.0 and 32.2 (2 × C-4), 39.8 and 39.9 (2 × CH_2N), 40.9 and 41.3

(2 × C-3), 46.5 (2 × C-2), 170.2 (CON), 204.8 and 204.9 (COS); *m/z* (ESI) 268 (MNa^+, 100%); Found (ESI): 268.1347, ($C_{12}H_{23}NO_2SNa$ requires 268.1342).

Ethyl 2,4-dimethylhexanoate 266[158]



A mixture of unsaturated esters **257** and **260** (0.50 g, 2.94 mmol) was dissolved in methanol (5 mL) and palladium on activated charcoal (≈ 20 mg) was added. The solution was stirred under an atmosphere of hydrogen (at a pressure of 7 bar) for 3 days. The catalyst was filtered off and washed with methanol. The solvent was then removed *in vacuo* to yield an approximately 1:1 mixture of the two diastereomeric pairs of saturated ester **266** (0.51g, 100%) as a colourless oil. δ_H(400 MHz) 0.83-0.90 (12H, m, 2 × 4-CH_3 and 2 × 6-H_3), 1.13 (10H, m, 2 × 2-CH_3 and 2 × 5-H_2), 1.25 (6H, t, *J* 7.0, 2 × OCH_2CH_3), 1.28-1.44 (4H, m, 2 × 3-*H*H and 2 × 4-H), 1.46-1.55 (1H, m, 3-H*H*), 1.72 (1H, ddd, *J* 13.5, 9.5, 5.0, 3-H*H*), 2.45-2.57 (2H, m, 2 × 2-H), 4.12 (4H, q, *J* 7.0, 2 × OCH_2); δ_C(100 MHz) 11.0 and 11.1 (2 × C-6), 14.2 (2 × OCH_2CH_3), 17.0 and 17.9 (2 × 2-CH_3), 18.8 and 19.0 (2 × 4-CH_3), 29.3 and 29.5 (2 × C-5), 32.1 and 32.3 (2 × C-4), 37.3 and 37.4 (2 × C-2), 40.6 and 41.1 (2 × C-3), 59.9 and 60.0 (2 × OCH_2CH_3), 176.9 and 177.2 (2 × C-1).

Chapter 7

Culture Work Experimental

Preparation of Glucose Deficient L-Agar Slants

Agar (3.00 g), ammonium sulfate (0.05 g), disodium hydrogen phosphate (0.26 g), glucose (0.11 g), monobasic potassium phosphate (0.24 g) and yeast extract (0.285 g) were mixed together and made up to 100 mL with water. The pH of the solution was adjusted to pH 7.0 with sodium hydroxide (1.0 M) prior to autoclave sterilisation at 121 °C for 15 minutes. The L-agar solution was then melted in the microwave and approximately 2 mL aliquots were poured into sterile tubes which were then allowed to set on their side at a gentle slope.

Preparation of Nutrient Broth

Glucose (2.00 g) and nutrient broth no. 2 (3.00 g) and were mixed together and made up to the 100 mL with water. The pH of the solution was adjusted to pH 7.0 with sodium hydroxide (1.0 M) prior to autoclave sterilisation at 121 °C for 15 minutes.

Rehydration of *Pseudomonas fluorescens* NCIMB 10586

A freshly opened lyophile of *P. fluorescens* NCIMB 10586 was rehydrated by suspension in sterile nutrient broth (0.5 mL). The suspension was deposited in sterile nutrient broth (4.5 mL) with stirring to ensure homogeneity. 10 µL aliquots of this suspension were then pipetted onto the surface of the glucose deficient L-agar slants and spread over the surface with a sterile inoculating loop. These were left to grow at 25 °C for 48 hours and were then stored in the fridge at 4 °C. Under these conditions the culture was viable for approximately 3 months. In accordance with the instructions issued with the lyophile, the bacteria was subcultured twice prior to use.

Preparation of Glycerol Stock Solution

A freshly opened lyophile of *P. fluorescens* NCIMB 10586 was rehydrated by suspension in sterile nutrient broth (0.5 mL). The suspension was deposited in sterile nutrient broth (4.5 mL) with stirring to ensure homogeneity. This solution was used to inoculate an L-agar plate (see below) and was allowed to grow for 24 hours at 25 °C. A single colony from the plate was used to inoculate a flask of primary stage medium (see below) and this was shaken at 250 rpm at 25 °C for 24 hours. Meanwhile a solution of glycerol (40 mL) was made up to 100 mL with water and sterilised by autoclave at 121 °C for 15 minutes. Equal measures of the glycerol solution and the

primary stage medium were then mixed together and transferred to vials for storage at -80 °C.

Preparation and Inoculation of L-Agar Plates

Agar (1.50 g), glucose (0.10 g), sodium chloride (1.00 g), tryptone (1.00 g) and yeast extract (0.50 g) were mixed together and made up to 100 mL with water. The pH of the solution was adjusted to pH 7.0 with sodium hydroxide (1.0 M) prior to autoclave sterilisation at 121 °C for 15 minutes. The L-agar solution was then melted in the microwave and poured into sterile petri dishes which were allowed to cool and set for 20 minutes. A slope was flooded with 5 mL of nutrient broth and the bacteria was suspended in this by gently scraping the surface with a sterile inoculating loop. (Alternatively a glycerol stock solution from the freezer was used to inoculate the plates.) The loop was then repeatedly dragged across the surface of the L-agar plate in a series of short lines, turning the plate in such a way as to dilute the concentration of bacteria each time (Figure 33). The plates were left to grow at 25 °C for 24 hours and then stored in the fridge at 4 °C.

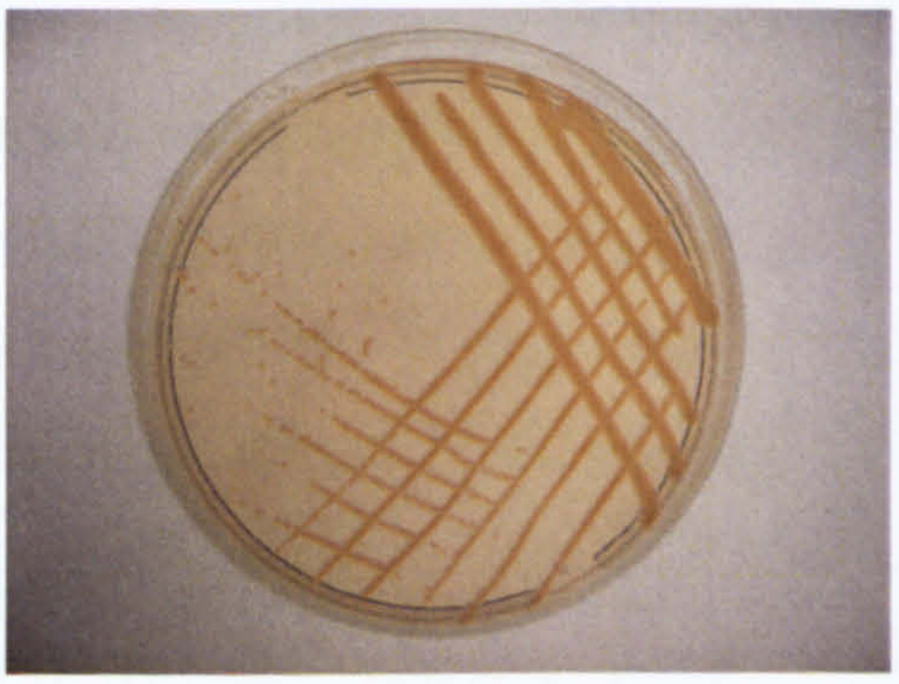


Figure 33: L-agar plate inoculated with *Pseudomonas fluorescens* NCIMB 10586.

Preparation and Inoculation of Primary Stage Medium

Glucose (0.10 g), sodium chloride (1.00 g), tryptone (1.00 g) and yeast extract (0.50 g) were mixed together and made up to 100 mL with water. The pH of the solution was adjusted to pH 7.0 with sodium hydroxide (1.0 M). 25 mL measures of media were then poured into 4 × 250 mL flasks which were bunged and capped with tin foil prior to autoclave sterilisation at 121 °C for 15 minutes. Single colonies of bacteria were then selected from the L-agar plate using sterile tooth picks, which were then

placed in each flask. The flasks were shaken at 250 rpm for 24 hours at 25 °C. The solution became cloudy indicating bacterial growth (Figure 34).

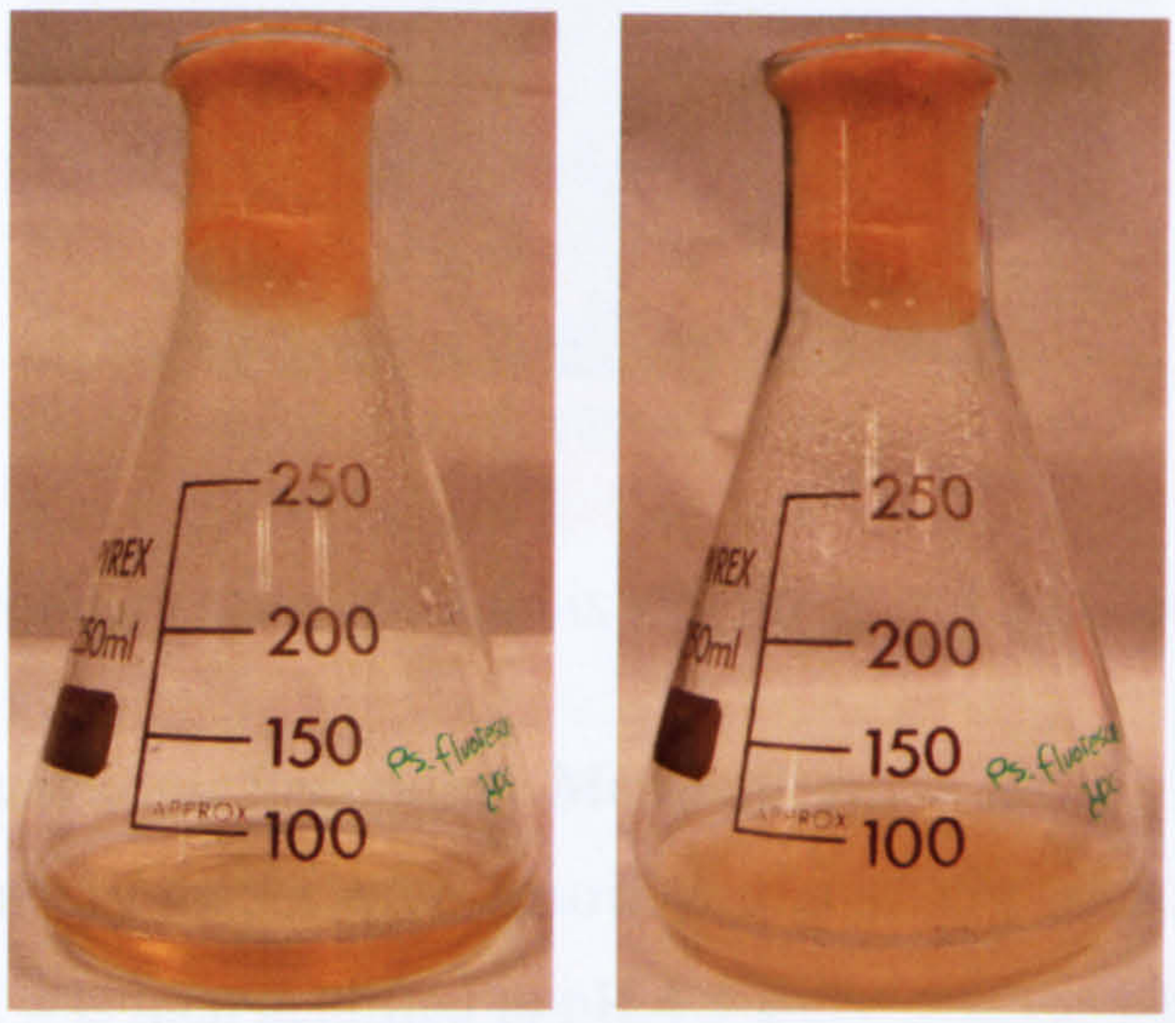


Figure 34: Primary stage medium before inoculation (left) and after 24 hours growth (right).

Preparation and Inoculation of Secondary Stage Medium

Ammonium sulfate (10.00 g), calcium carbonate (13.50 g), spray dried corn steep liquor (5.00 g), disodium hydrogen phosphate (2.00 g), magnesium sulfate heptahydrate (1.00 g), monobasic potassium phosphate (3.00 g), potassium chloride (2.00 g) and soya flour (40.00 g) were mixed together and made up to 1500 mL with water. Glucose (80.00 g) was dissolved in water and made up to 500 mL. The pH of both solutions was adjusted to pH 7.0 with sodium hydroxide (1.0 M) prior to autoclave sterilisation at 121 °C for 15 minutes. 20 × 500 mL baffled flasks were bunged and capped with tin foil and were also sterilised by autoclave. The glucose solution and primary stage medium (100 mL) were then added to the rest of the medium and stirred vigorously. 105 mL aliquots were then transferred to each of the 20 flasks with a sterile graduated cylinder. The flasks were shaken at 250 rpm for 50 hours at 25 °C (Figure 35).

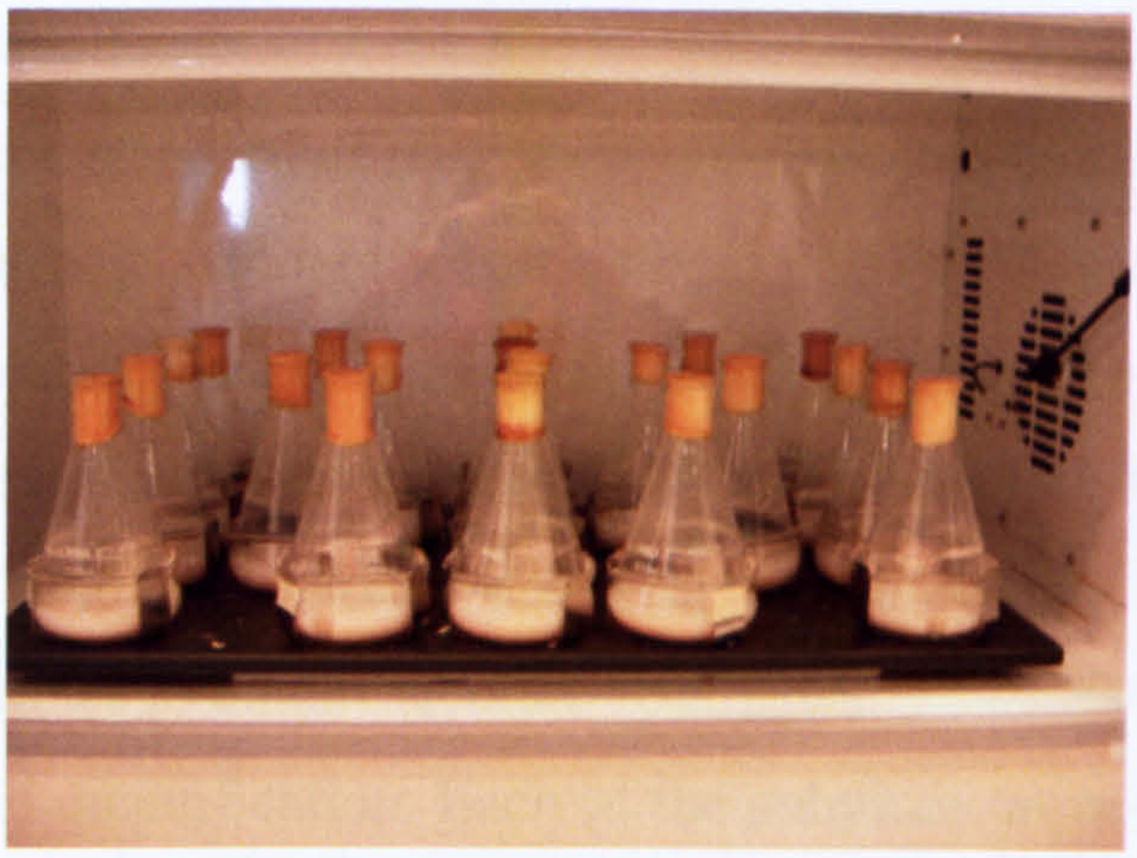


Figure 35: The secondary stage medium is shaken at 250 rpm for 50 hours at 25 °C.

Extraction of *P. fluorescens* Secondary Metabolites

The combined secondary media were centrifuged at 10,000 rpm for 15 minutes at 4 °C. The supernatant was then acidified to pH 4.5 and extracted with ethyl acetate (3 × 1,200 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo* to yield the crude extract as an orange-brown oil.

HPLC Analysis

A 50 µL sample of supernatant was analysed by HPLC using the conditions set out below (Table 3). Under these conditions, pseudomonic acid A has a retention time of approximately 21.5 minutes.

Column:	Luna C_{18} (5µ, 4.6 × 250 mm, Phenomenex)
Mobile Phase A:	Water, containing 0.005% formic acid
Mobile Phase B:	Acetonitrile, containing 0.005% formic acid
Mobile Phase System:	0-1 minutes: 95% A, 5% B 1-2 minutes: ramp to 63% A, 37% B 2-4 minutes: 63% A, 37% B 4-20 minutes: ramp to 1% A, 99% B 20-23 minutes: 1% A, 99% B 23-25 minutes: ramp to 95% A, 5% B 25-29 minutes: 95% A, 5% B
Flow Rate:	1 mL min^{-1}
Detector Wavelength:	233 nm

Table 3: HPLC conditions.

Quantification of Pseudomonic Acid A

A 1 mg/mL volumetric standard of mupirocin was prepared using a 25 mL volumetric flask. 25 mg of mupirocin standard was dissolved in a small quantity of HPLC grade methanol and the solution was then made up to the mark with methanol. 12.5 mL of this solution was then pipetted into a second 25 mL volumetric flask and made up to the mark with methanol to prepare a 0.5 mg/mL solution. This procedure was repeated a further 6 times to obtain accurate volumetric solutions in the range required for pseudomonic acid quantification. Each of the solutions were then run in duplicate by HPLC and an average peak area for each solution was calculated (Table 4). The data was then correlated into a linear graph from which samples of unknown concentration could be determined (See Figure 9, Chapter 2).

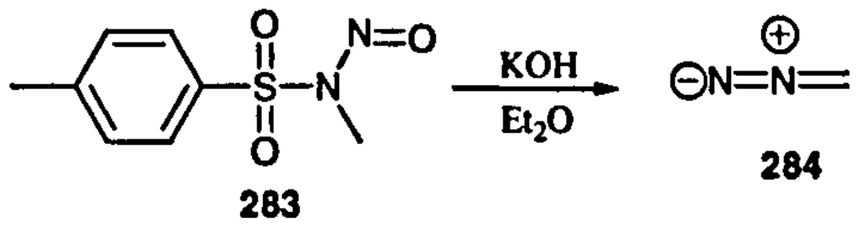
Standard Concentration (mg/mL)	Average Peak Area (mAu*min)
0.0078	5.0031
0.0156	11.2256
0.0313	21.8234
0.0625	45.5028
0.1250	87.1284
0.2500	174.6765

Table 4: HPLC values obtained for volumetric standards.

Growth Production Study

Samples of the fermentation broth were taken every two hours, starting ten hours after inoculation of the secondary stage medium. 1 mL aliquots were taken from two flasks at each interval, combined, micro-centrifuged and then the supernatant was analysed immediately by HPLC. The concentration of each sample was determined by comparison with the volumetric solution data and the pH of each solution was also measured (Table 5).

Preparation of Ethereal Diazomethane 284[175]



Caution: This reaction is potentially explosive and both reagents and products are carcinogens. For safety, ground glass joints must be avoided, acetic acid should be kept close by, heavy nitrile gloves should be worn and the fume cupboard should be

kept closed as much as possible. Potassium hydroxide (0.33 g, 5.89 mmol) was dissolved in water (2 mL) and diluted with ethanol (3 mL). This was then placed in the reaction flask (for safety reasons a preassembled distillation apparatus, containing no ground joints was used and acetic acid was kept beside the reaction flask at all times) and cooled to 0 °C. A solution of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide **283** (1.25 g, 5.83 mmol) in diethyl ether (5 mL) was added dropwise. When addition was complete the ice bath was placed under the receiver flask and a water bath was used to warm the contents of the reaction flask to boiling. When the yellow diazomethane-ether mixture had finished distilling across and the reaction mixture had become colourless, the distillation was deemed complete. The product was used immediately.

Sample Time	pH Supernatant	Area Pseudomonic Acid (mAu*min)	Conc. Pseudomonic Acid (mg/mL)
10	5.67	7.9016	0.0111
12	5.55	18.7501	0.0263
14	5.37	31.9608	0.0447
16	5.49	46.3093	0.0648
18	5.27	68.4713	0.0959
20	5.39	66.8376	0.0936
22	5.35	81.3070	0.1138
24	5.22	98.4708	0.1379
26	5.16	85.5693	0.1198
28	5.18	102.0936	0.1429
30	5.11	89.9922	0.1260
32	5.04	93.1250	0.1304
34	5.00	85.8140	0.1201
36	5.12	72.2774	0.1012
38	5.45	84.0376	0.1177
40	6.11	75.8213	0.1061
42	6.53	94.2643	0.1320
44	6.48	79.1372	0.1108
46	7.09	95.3378	0.1335
48	6.96	113.3893	0.1587
50	7.26	89.3494	0.1251
52	7.25	115.4894	0.1617
54	7.55	94.0669	0.1317
56	7.78	88.8304	0.1244

Table 5: Results of growth production study.

Methylation of *P. fluorescens* Extracts and Isolation of Methyl Pseudomonate

Extracts of *Pseudomonas fluorescens* NCIMB 10586 were stirred in methanol, cooled to 0 °C and treated dropwise with an excess of ethereal diazomethane **284.** The solution was stirred for 10 minutes and then the excess diazomethane was blown off with nitrogen. The resulting residue was then purified by column chromatography (Sephadex®, 100% methanol; SiO_2, 50-100% ethyl acetate/petroleum ether 40-60 °C) to afford methyl pseudomonate (0.04 g) as a white solid. For spectral data see Section 2.4, Figure 12 and Figure 13).

Feeding Study With [1,2-$^{13}C_2$]-9-hydroxynonanoic *N*-Acetylcysteamine Acid Thiol Ester 1

Primary and secondary media were prepared and inoculated as described above. A solution of thiol ester **1** (60 mg) in ethanol (≈1-2 mL) was diluted with water (≈48-49 mL) and sterilised by autoclave at 121 °C for 15 minutes. 1 mL aliquots of this solution were transferred to each of 10 secondary stage media flasks 10 hours after inoculation. This was repeated hourly for a further 4 hours. The cells were harvested, methylated and purified as described above and 18 mg of methyl pseudomonate (slightly contaminated with an unknown impurity) were isolated. The ^{1}H and ^{13}C NMR spectra were identical to authentic material.

Feeding Study With [3,3-$^{2}H_2$]-3-hydroxypropanoic *N*-Acetylcysteamine Acid Thiol Ester 2

Primary and secondary media were prepared and inoculated as described above. A solution of thiol ester **2** (100 mg) in ethanol (≈1-2 mL) was diluted with water (≈48-49 mL). 1 mL aliquots of this solution were transferred to each of 10 secondary stage media flasks 10 hours after inoculation. This was repeated hourly for a further 4 hours. The cells were harvested, methylated and purified as described above. No methyl pseudomonate was isolated from the feed flasks nor from the control flasks.

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